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# CONTENTS

## No. 1 MARCH, 1928

Maynard M. Metcalf. Trends in evolution: A discussion of data bearing upon 'orthogenesis.' Sixty-one figures ... 1
Josephine Willis McNabb. A study of the chromosomes in meiosis, fertilization, and cleavage in the grasshopper egg (Orthoptera). Nine plates (sixty-six figures) ... 47
D. L. Hopkins. The effect of certain physical and chemical factors on locomotion and other life processes in Amoeba proteus. Four figures ... 97
Rudolf G. Schmieder. Observations on the fat body in Hymenoptera. Six plates ... 121
Harold Heath. A sexually mature turbellarian resembling Müller's larva. Sixteen figures ... 187
David S. Pankratz. The cranial musculature of the toadfish (Opsanus tau). Eleven figures ... 209
Hope Hibbard. Cytoplasmic constituents in the developing egg of Discoglossus pictus Otth. Sixteen figures ... 233
G. K. Noble and M. E. Jaeckle. The digital pads of the tree frogs. A study of the phylogenesis of an adaptive structure. Eighteen figures ... 259
A. A. Schaeffer. Spiral movement in man. Ten figures ... 293

## No. 2 JUNE, 1928

Mabel A. Myers. A study of the tonsillar developments in the lingual region of anurans. Twenty-six figures ... 399
Enid Oldham Lapage. The septomaxillary. I. In the Amphibia urodela. Seven plates (twenty figures) ... 441
Cecil R. Monk. The anatomy and life-history of a fresh-water mollusk of the genus Sphaerium. Twenty figures ... 473
Henry P. Dorman. The morphology and physiology of the hepatopancreas of Cambarus virilis. Seventeen figures ... 505
Henry P. Dorman. The morphology and physiology of the invertebrate liver and hepatopancreas. Nine figures ... 537
Frederick E. Emery. The metabolism of amino-acids by Paramecium caudatum. Three figures ... 555
Edith Pinney. Developmental factors in teleost hybrids. I. The early mitotic behavior in the cross between Menidia menidia notata ♀ and Prionotus carolinus ♂. Two plates (twelve figures) ... 579
Pauline H. Dederer. Variations in chromosome number in the spermatogenesis of Philosamia cynthia. Two plates (twenty-seven figures) ... 599

# TRENDS IN EVOLUTION: A DISCUSSION OF DATA BEARING UPON 'ORTHOGENESIS'

MAYNARD M. METCALF

SIXTY-ONE FIGURES

AUTHOR'S ABSTRACT

Five characters—flatness elongation, posterior pointedness, delay in division of the body, delay in completion of mitosis—are so distributed among the subdivisions of the Opalinidae as to involve either repeated fortuitous appearances of these characters, a thing not to be believed, or trends resident in the germ plasm. The Ophryoscolecidae show similar distribution of two sets of characters In the Salpidae there is evidence of trends toward coiling of the gut, decrease in number, size, regularity, and symmetry of body muscles, simplification and degeneration of the eyes These qualities appear first in the phylogeny in the chain Salpas, the final phase in the life cycle. In the course of the evolution the solitary Salpas become more and more modified in the same directions, until in the most highly modified species, the muscles and eyes are as much modified in the solitary Salpas as in the aggregated These changes, not disadvantageous but rather adaptive in their beginning in the colonial individuals, are harmful to the solitary Salpas, yet the degeneration is, by precocious development, thrown back onto the earlier phase of the life cycle, the solitary stage. Precocious development is not purely utilitarian, but may be more fundamental, biological

Evolution is discussed in terms of trends resident in the germ plasm, their origin in connection with mutations, their growth, decrease, disappearance, branching, auto-evolution Discussion is from the standpoint of the germinal stream, internal factors of evolution being emphasized.

## INTRODUCTION

The purpose of this paper is not so much to present conclusions as to develop a point of view to be held in mind and tested by observation and experiment, and especially to emphasize a method of testing the presence of evolutionary trends, in different groups of organisms, apart from environmental control. Phenomena among the Opalinidae, the Ophryoscolecidae and the Salpidae will be presented.

In the light of these phenomena, evolution will be treated more from the standpoint of the germinal stream and its branches and less than is customary from the standpoint of the individual organism. The germinal stream is the persistent, the essential, the fundamentally important thing. Individual organisms are but transient flecks of foam upon the surface of this stream. Evolution has generally been discussed from the standpoint of the relation of organisms to their environment and from the point of view of the selec-

JOURNAL OF MORPHOLOGY AND PHYSIOLOGY, VOL. 45, NO. 1
MARCH, 1928

tive reaction of the environment upon individual organisms. We shall attempt to consider the process and progress of evolution in the germinal stream itself, in the stirp.[1] From this point of view, individual organisms are of interest chiefly because of what they tell us as to the stirp and as to its probable behavior. We shall dwell more upon the internal factors in evolution, those shown within the organism, in 'the nature of the beast,' and will give less thought to the regulatory action of the environment through its selective pressure upon the individual, although, of course, this is always present and is the ultimate check, determining survival for both the individual and the stirp.

Evolution is dependent upon changes in the germinal plasm, usually called mutations; upon the appearance of new qualities, their increase, their decrease, or their disappearance again. I wish to note certain phenomena, chiefly in the three groups of organisms named above, which suggest that some characters which appear as a result of germinal change, of mutation, increase from generation to generation, apparently without environmental aid, seeming to increase because of a progressive germinal change which when once started may go on to greater and greater development because of conditions in the germ plasm itself. We will note indications that such trends, inherent in the stirp itself, may wax or wane, i.e., first increase and then decrease and even disappear. We will note in the Salpidae how two such trends may have arisen.

The Opalinidae are a peculiarly favorable group for use in study of trends and the behavior of trends. They are ciliate Infusoria commensal in the recta of Anura and live secluded lives in a very uniform environment. Probably more than most organisms, they escape much of the stress of the struggle for existence. The ratio of importance between internal and external factors in the evolution of such organisms as the Opalinidae is probably more in favor of the internal factors than is the case among free-living organisms, whose environment is more varied. Such free-living organisms have more opportunity to gain advantage for themselves by developing adaptations to special conditions occurring in their varied environment. Also, free-living organisms are exposed to a larger series of stimuli which might instigate mutation. Such evidence of trends, therefore, as is furnished by such secluded forms as the Opalinids and the Ophryoscolecids, both of which are internal commensals, may well be given especial consideration. The Salpidae also, living all their lives, as they do, floating in the open ocean, meet a less diverse set of environing conditions than do terrestrial or littoral organisms; so in the Salps, as in the internal parasites and commensals, there is less opportunity for gaining further advantage through special adaptations, when once the main adjustment to the relatively uniform conditions of pelagic life has been acquired. All such forms are removed from some of the stress of the struggle for existence and from some of the opportunities for survival through further special adaptation, which a more varied environment would present. External factors of evolution probably play among them a less important rôle, the internal factors being relatively more important.

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[1] This word is used to mean the branch of the germinal stream under consideration.

The Opalinidae, the most archaic family of ciliate Infusoria,[2] show with remarkable clearness the taxonomic interrelation between the four genera of the family, and the taxonomic relations between certain subgenera are clear. The evidence for these statements cannot well be presented within the limits of this paper. The accompanying table of classification and the phylogenetic chart (fig. 1 and fig. 2) state conclusions drawn from data already published (Metcalf, '23).

*Protoopalina* is the most archaic genus of the family, its most primitive subgeneric group, including species from South Africa, Patagonia, and Australia, all having the posterior end of the body drawn out into a slender, nonciliated point (fig. 3). *Protoopalinae* are circular, or nearly so, in cross-section and they have two nuclei, either distinct from each other or united together by a slender thread persisting from the last (incomplete) mitosis (fig. 4). The binucleated condition is less established than in the Euciliata, being but temporary, since the gametes are generally uninuclear, being formed by fission from binucleated individuals. In fission in *Protoopalina,* whether transverse or longitudinal, each daughter cell receives the whole of one of the two parent nuclei, whereas in Euciliata each daughter cell receives one-half of each parent nucleus. The Euciliata show true binucleation, while *Protoopalina* is only pseudobinucleate. Its binucleation is due to temporary suppression of division of the body, although the nucleus has completed or nearly completed its mitosis, giving thus a temporary condition of two distinct or nearly distinct nuclei in one body.

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[2] They are evidently relicta from an early period of the evolution of the Ciliata, secondarily modified in connection with their habit of internal commensalism.

CILIATA

PROTOCILIATA

- Opalinidae
  - *Protoopalininae* (binucleated)
    - *Protoopalina* (spindle-shaped)
      - (nine subgenera)
    - *Zelleriella* (flat)
  - *Opalininae* (polynucleated)
    - *Cepedea* (spindle-shaped)
      - (six subgenera)
    - *Opalina* (flat)
      - *Opalinae latae* (broad forms, arose in Eastern Hemisphere)
      - *Opalinae angustae* (narrow forms, arose in Western Hemisphere)

EUCILIATA

Fig. 1 Classification of the Opalinidae.

*Protoopalina* shows not only a habit of delaying body division; it has also a habit of delaying completion of mitosis, the resting phase of the nucleus not showing the reticulate or granular arrangement of chromatin characteristic of resting nuclei of other animals and plants. The 'resting' nuclei of *Protoopalina* are in one or another midmitotic phase.

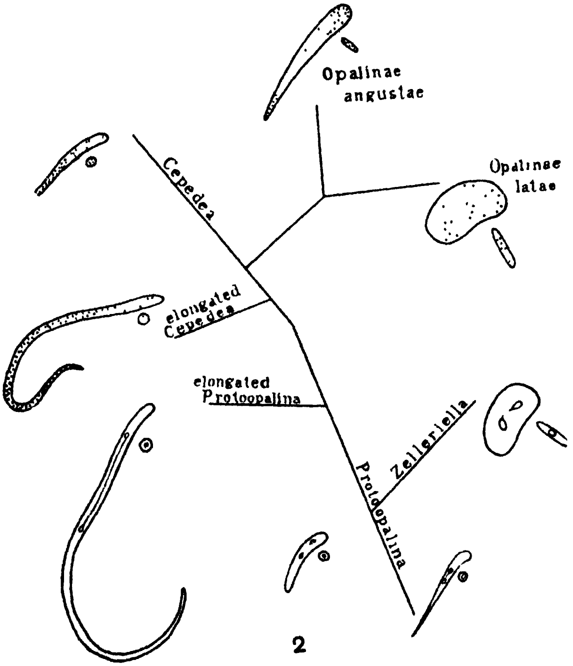
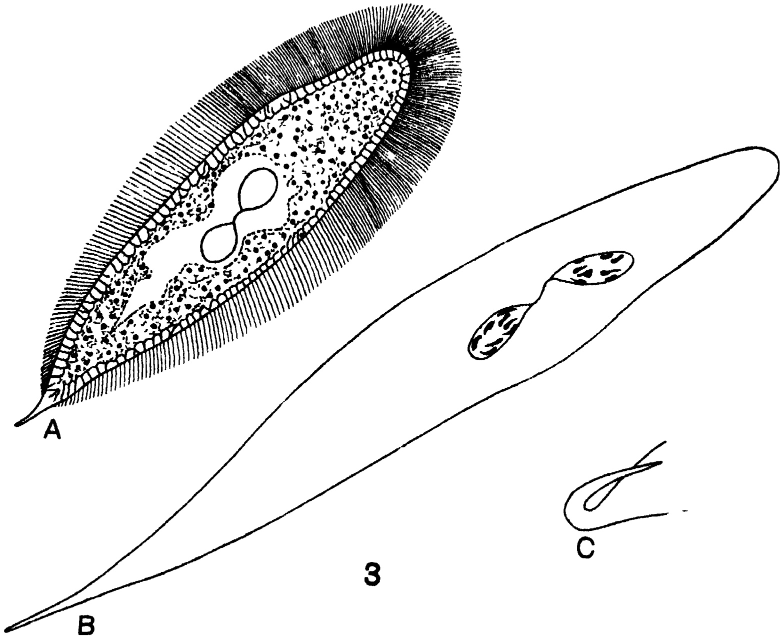


Fig. 2 Phylogeny of the Opalinidae.

Many a species of *Protoopalina* shows its nucleus not completely divided into two, the two daughter nuclei being still connected by a hollow thread (fig. 4). In the several species the midmitotic phase in which the dividing nucleus comes to 'rest' is characteristic of the species. Observe a series of drawings showing the characteristic 'resting' condition in a number of species: figure 5, ***Protoopalina rhinodermatos,*** resting nuclei granular—a very unusual resting condition in



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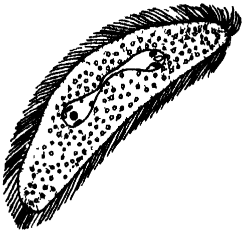

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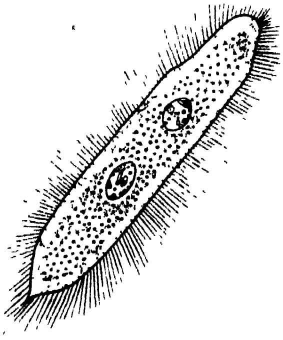

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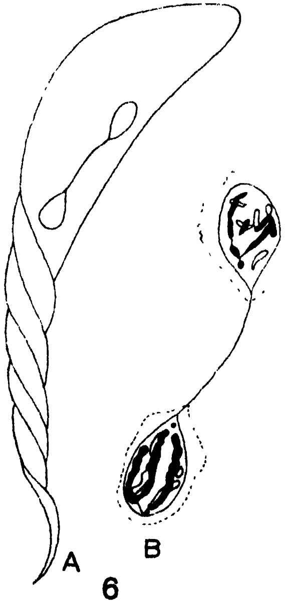
Fig. 3 *Protoopalina xenopodos*: A, an ordinary individual; B, an elongated individual ready for fission; C, the posterior end of a third individual.

Fig. 4 *Protoopalina montana.*

Fig. 5 *Protoopalina rhinodermatos*, 'resting' condition, nuclei with granular chromatin, a condition seldom found in this genus in 'resting' nuclei.

*Protoopalina.* Figure 6, A, *P. papuensis;* figure 6, B, nuclei from the same individual, showing a double-skein condition. The anterior nucleus is slightly more advanced in its mitotic phase, as is not unusual in *Protoopalina.* Its chromatin thread is breaking into separate chromosomes. Figure 7, *P. filiformis,* with nuclei roughly in an equatorial plate condition. The equatorial plate phase of mitosis is usually rather indefinite in the Opalinidae. Figure 8, *P. regularis,* with early anaphase nuclei. Figure 9, *P. hammondii,* with late anaphase nuclei. Figure 10, *P. scaphiopodos,* with early telophase nuclei. Figure 11, *P. formosae,* with very late telophase nuclei, the four daughter nuclei being almost dis-



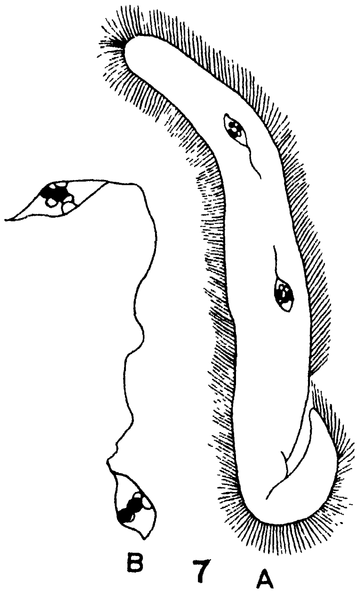
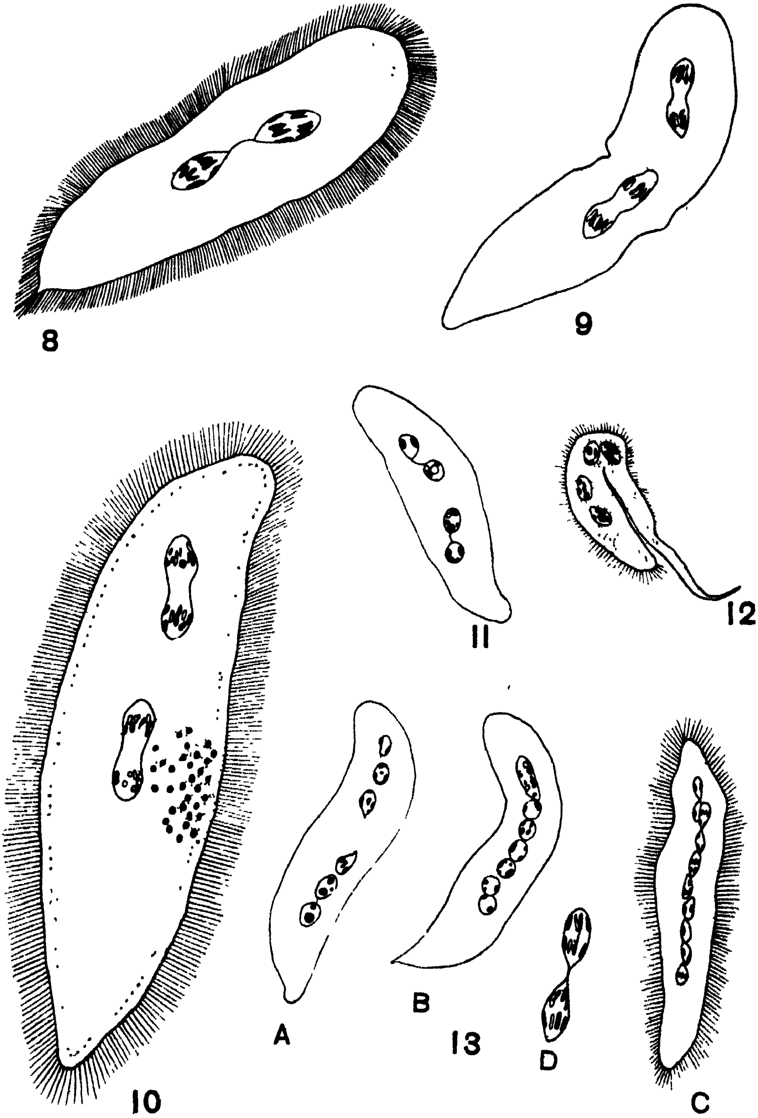


**Fig. 6** *Protoopalina papuensis*: A, an ordinary individual; B, its 'resting' nuclei with chromatin in a skein condition, just beginning to break up into chromosomes.

**Fig. 7** *Protoopalina filiformis*: A, an ordinary individual; B, its nuclei more highly magnified.



Figures 8 to 13

tinct. Figure 12, *P. quadrinucleata*, with four distinct nuclei. Figure 13, *P. axonucleata*, which usually has from six to ten nuclei. One can find in any species of *Protoopalina* individuals with nuclei in any phase of mitosis, but the characteristic phase of the nuclei in 'resting' individuals, that is, in individuals not active in mitosis, is different in different species. In other words, *Protoopalinae* do not complete promptly a mitosis once entered upon, but come to rest with their nuclei in some midmitotic phase of chromatin arrangement; and different midmitotic phases are specifically characteristic of the 'resting' nuclei in different species. This series of drawings (figs. 4 to 13) illustrates, first, some specifically characteristic, midmitotic, 'resting' phases in *Protoopalina* and, secondly, delay in division of the body until after the completion of from one-half to three mitotic cycles.

Delay of body fission is a generic character in *Protoopalina*, giving pseudobinucleation. Also characteristic of the genus is delay in completing mitosis, and the characteristic degree of this delay in mitosis, as shown in the midmitotic phase of the 'resting' nucleus, is a character of diagnostic value for the several species.

*Protoopalina* gave rise to the genus *Cepedea*, partly by still further delay in body division, more than one division of the body being temporarily suppressed, giving not a pseudobinucleate, but a pseudopolynucleate condition (figs. 14 and 15). However, before conjugation, rapid divisions of the body, with little increase in nuclear division, gives uninucleated gametes. In *Cepedea* the nuclei 'rest' with the chromatin in a reticulate condition so that resting individuals of

**Fig. 8** *Protoopalina regularis*, with early anaphase nuclei.

**Fig. 9** *Protoopalina hammondii*, with late anaphase nuclei.

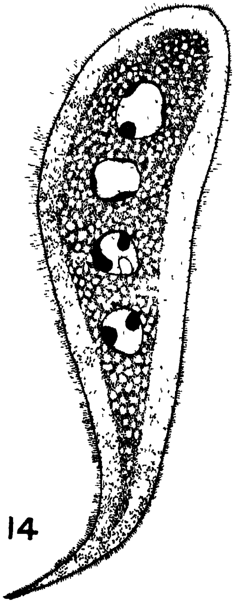
**Fig. 10** *Protoopalina scaphopodos*, with early telophase nuclei.

**Fig. 11** *Protoopalina formosae*, with very late telophase nuclei.

**Fig. 12** *Protoopalina quadrinucleata*, with four distinct nuclei.

**Fig. 13** *Protoopalina axonucleata*, which usually has from six to ten nuclei: A, with six nuclei; B, with seven nuclei, the seventh ready to divide to make eight; C, with ten nuclei; D, two nuclei, not yet separated, each in an anaphase stage of mitosis.

the several species do not show characteristically diverse nuclear conditions. But the species do show differing degrees of suppression of body divisions, as illustrated. That is, one species (*C. lanceolata*, fig. 14) has four nuclei, one (*C. spinifera*, fig. 15) about sixteen, others, say, forty or fifty (*C. formosae*, fig. 16) and so on up to species with several hundred nuclei (*C. magna*).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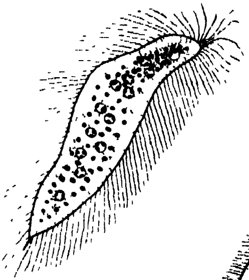
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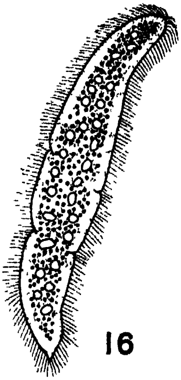
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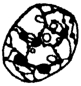
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Fig. 14 *Cepedea lanceolata* (after Bezzenberger, '04).
Fig. 15 *Cepedea spinifera.*
Fig 16 *Cepedea formosae.*
Fig. 17 A 'resting' nucleus of *Opalina obtrigonoidea*, only the upper portion being drawn.

The intergradation between *Protoopalina* and *Cepedea,* while complete in number of nuclei, is not so in nuclear condition. The nuclei in *Cepedea,* like those in *Opalina* (fig. 17), do not rest in a midmitotic condition as in *Protoopalina,* but have their chromatin reticulate and granular when not actively dividing. The nuclei of those species of *Protoopalina* which have four and eight nuclei are distinguishable at a glance from those of *Cepedea.*

We have noted for the genera *Protoopalina* and *Cepedea* some of the relations of delayed mitosis and delayed fission to speciation. There are two other qualities, of body shape, elongation and flattening, that bear relation to the speciation. *Protoopalina* is circular (or nearly so) in cross-section. From *Protoopalina* is derived another binucleated genus, *Zelleriella,* merely by flattening of the body (fig. 2).

The multinucleate genus *Opalina* arose from *Cepedea* by a similar process of flattening (fig. 2), so that both in the more primitive subfamily with binucleated species and in the derived subfamily with multinucleated species the more archaic genus is cylindrical, while the more modern genus is much flattened.

In the genus *Opalina* it is germane to our discussion to distinguish a subgeneric group of broad species, *Opalinae latae,* and a more modern group of narrow species, *Opalinae angustae,* derived from the former, apparently in North America late in the Tertiary period.

The phyletic relations here given between these genera and subgenera are indicated by their form and cytological structure and are confirmed, *a*) by their host distribution, related Opalinids being, in general, in related hosts; *b*) by their geographical distribution, related forms, of both hosts and parasites, being found in contiguous territory or in places to which they might naturally have spread by known routes, and, *c*) by their life-histories, as, for example, in the *Opalinae angustae,* some species, at least, of which ·in the frog tadpole are known to be, after conjugation, in appearance, at first *Protoopalinae,* becoming later *Cepedeae,* then *Opalinae latae,* and finally, *Opalinae angustae* (Metcalf, '24, '26), reaching the latter condition late in the tadpole life of its host or at the time of its metamorphosis, thus repeating in the course of the individual development the phyletic history of the group.

We see, thus, in the family Opalinidae several characteristics which by their presence or absence, their conjunction or disjunction, give the chief diagnostic features of the two subfamilies, of the genera, and of some of the subgenera. These characters mentioned are flatness, elongation, delay in division of the body, and delay in completion of mitosis. Pointedness of the posterior end of the body is another characteristic to be discussed later. It is the shuffling and distribution of these five characters in the family which give us the main features of the several subdivisions of the family (subfamilies, genera, subgenera) (fig. 18).

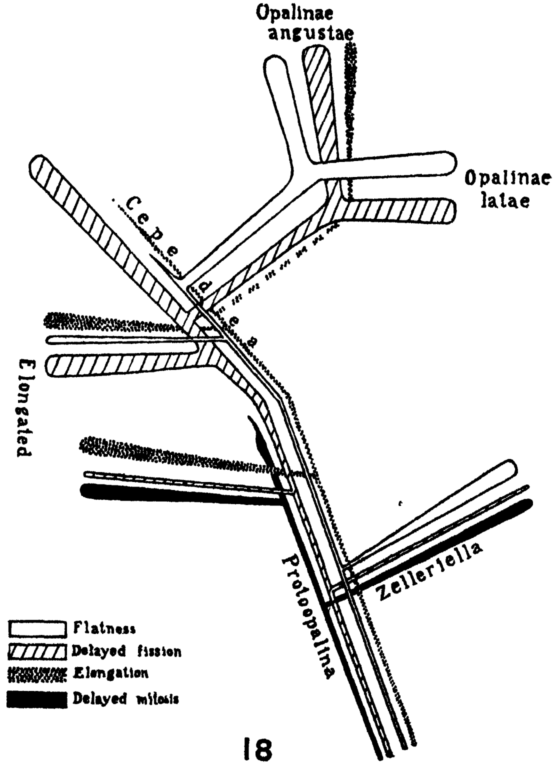


Fig. 18 Chart of the distribution of four characters in the family Opalinidae.

This, as thus far described in outline, is all introductory to the point of special interest in our discussion. As we observe the distribution of these characters along the stem and branches of the Opalinid family tree, we see some of these characters increase, others decrease or even disappear, some increase independently in separate branches, and some increase, then decrease and lie latent, to increase again later in a more distal branch. As one thinks of these conditions, he sees two alternatives in explanation: either there are instances of independent appearance of the same sort of mutation (character), or else these several appearances of the same sort of mutation are not independent of one another, but are rather each dependent upon some condition in the family stirp, this inherent potentiality producing a developed character at more than one point of the phyletic history. A simple and appropriate term for expressing this flair for repeated development of an inherent potentiality into a discernible character seems to be the word 'trend.' Independent appearances of the same sort of mutation, for example, delay in fission, seem very improbable. The existence of inherent trends seems much more probable. We may speak then, 1) of a trend toward flattening; 2) of a trend toward elongation; 3) of a trend toward delay in the division of the body; 4) of a fourth trend toward delay in the completion of mitosis, and 5) of at least one other trend, posterior pointedness.[3] A potentiality, weakly expressed, though present, in the ancestral stock, which is likely to become more emphasized at different points in the later phyletic development (as the phyletic history shows) we shall call a trend.

Let us now note in the Opalinidae each of the five trends named. First note the tendency to *flatness*. This is present in the whole family and becomes emphasized more than once in the course of the phyletic development. In some of the species of the most archaic subgenus of the most archaic genus, *Protoopalina,* the body is noticeably flattened on what Rapp ('11) calls the 'ventral' surface. More modern species of the genus are for the most part little or not at all flattened. But some of the most developed species again are considerably flattened. In the case of two of these species one is a bit doubtful whether to call them unusually flattened *Protoopalinae* or unusually stocky *Zelleriellae. Zelleriella* is a genus which arose (in South America, probably in the early Tertiary) from *Protoopalina,* by a flattening of the body. In most species this flattening is extreme, the whole thickness of the body being little more than that of one of the nuclei. The genus *Cepedea* arose from *Protoopalina* (apparently in southern India in Jurassic times) by multiplication of nuclei unaccompanied by fission. A number of species of *Cepedea* are somewhat flattened anteriorly, the anterior end being wedge-shaped. Some species, especially some of the most elongated ones, are considerably flattened throughout the body. Other species are so much flattened that one is in some doubt whether to call them *Cepedea* or *Opalina.* The genus *Opalina* arose from *Cepedea* (apparently in southeastern Asia during the Cretaceous period) by flattening of the body. Except for a very few species, perhaps survivors from an intermediate condition, the *Opalinae* are extremely flattened, as much so as is *Zelleriella.*

---

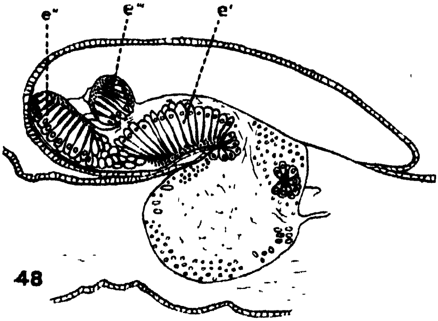
[3] The distribution of these characters in the family Opalinidae and the evidence thus given of trends in the evolution of the family were discussed before the American Society of Naturalists, at Cincinnati, Ohio, December 29, 1923: "Inherent trends versus natural selection in the evolution of parasites."

We thus see at least some flattening, 1) in the earliest *Protoopalinae,* the most archaic members of the family; 2) in some members of each of several other subgenera of *Protoopalinae;* 3) in *Zelleriella;* 4) in the elongated *Cepedeae;* 5) in some *Cepedeae* approaching *Opalina* in form, and, 6) in all the *Opalinae.* No phylogenetic tree, not preposterous, could be so drawn as to avoid assumption of emphasis upon flattening at several points in the phyletic development of the family. In general, the flatter species are among the more aberrant members of the family.

Another simple character of shape, *elongation,* is moderately developed in most *Protoopalinae,* but is greatly emphathe eye disappear altogether, e.g., the dorsal plug, (figs. 43, 44, 48, 49, 50, 51, 52, 53, 54, 56). At the same time the rod cells lose their regularity of shape and arrangement (figs. 48, 55, 56), their terminal rods are lost and in place we find the whole 'rod cell' wall showing merely irregular glassy thickening (fig. 58, compare fig. 57). Even in the solitary forms of some species the rod cells become degenerate (fig. 47). Somewhat similar changes might be traced for certain accessory eyes present in the brain in the aggregated zooids of different species, but it seems hardly best to include here the descriptions needed to make this clear. The things to emphasize are, first, the progressive degeneration of the eyes and, secondly, that in these phylogenetic changes the aggregated zooids lead and the solitary Salps follow.

The Salpas show thus three trends: 1) toward coiling of the gut; 2) toward simplification and irregular arrangement of the body-muscle series, and, 3) toward degeneration of the eyes.

We have thus far noted the presence of five trends in the family Opalinidae, of two in the Ophryoscolecidae, and of three in the Salpidae. In the case of the Salpidae we find some indication of how two of these trends may have started. I see no clear suggestion as to the origin of the trend toward compact coiling of the gut, except that in the phylogeny of the Salpidae the exhalent aperture has been drawn backward from an originally more dorsal position, such as is seen in the ascidians, and that it might be advantageous for the anus also to move back so as to be near the exhalent siphon. But the degeneration of the eyes and of the muscle system may well have been permitted by the habit of the aggregated individuals of living in a chain. The solitary Salps have to fend each for itself. But the aggregated zooids live most of their lives as members of a more or less cooperative community, the 'chain.' The young chain individuals are carried by the solitary Salp and have little need of muscles or eyes of their own. Only after they reach their full development, except for size, are they freed from the chain, and then a number of



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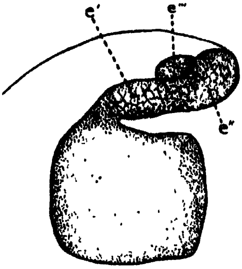

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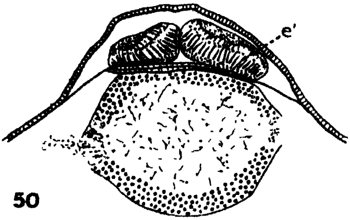

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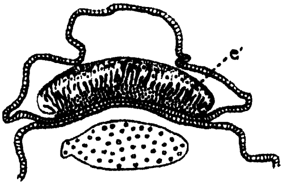

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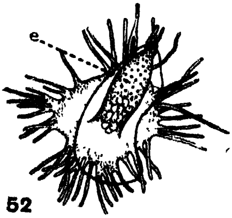

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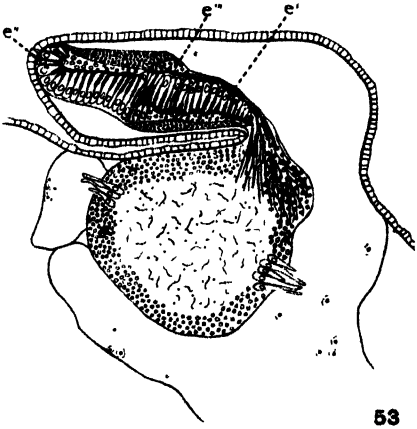

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Figures 48 to 53

individuals usually break away together forming quite a long colony, and they continue bound together, though no longer attached to the solitary Salp. Thus, during the whole period of their development and usually until sexual reproduction is accomplished, they are members of a cooperative colony. Because of the mutual aid between individual zooids, there is less need for each zooid to have a full and perfectly developed series of muscles or perfect eyes. Natural selection does not operate so keenly upon these cooperating zooids in respect to their muscles and eyes.

But this is not all that is involved. Life in a chain group does not merely permit departure from the ancestral arrangement and structure of muscles and eyes; it goes further and promotes changes in these organs. A word about the arrangement of the zooids in the chain is necessary to make the matter clear. In the *Salpa* chain, as indeed throughout the Tunicata, bud-formed individuals arise with the dorsal surface toward the parent and the ventral surface away from the parent; belly to back is the position. On the *Salpa* chain many buds are serially formed by constriction of the stolon, the oldest bud at the tip of the stolon and the successively younger buds forming in series toward the base of the stolon. In the young stolon the bud zooids lie in a linear series belly to back. But, as the zooids increase in size and crowd one another, they change their relative positions, becoming in most species apparently a double row of zooids, though not really so in fundamental relations. This is brought about by the zooids shifting alternately to the right and to the left, no.

**Fig. 48** A parasagittal section of the eye and brain of *Salpa* (*Cyclosalpa*) *pinnata*, aggregated form. In this figure the anterior end is at the left, the reverse of figure 44.

**Fig. 49** The eye and brain of *Salpa* (*Cyclosalpa*) *affinis*, aggregated form, seen from the right side.

**Fig. 50** *Salpa* (*Cyclosalpa*) *pinnata*, aggregated form. A transverse section of the posterior portion of the eye (the brain also showing).

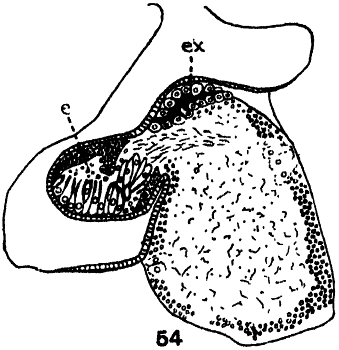
**Fig. 51** A similar section from *Salpa* (*Cyclosalpa*) *affinis*.

**Fig. 52** *Salpa* (*Cyclosalpa*) *virgula*, aggregated form. The eye and brain.

**Fig. 53** A sagittal section of the same.

Lettering as in figures 42 to 47.

1 to the right, no. 2 to the left, no. 3 to the right, and so on. The result is a double row of diagonally placed individuals (compare Brooks, '93) in which one side of each zooid is more cramped than the other side. The zooid that has shifted to



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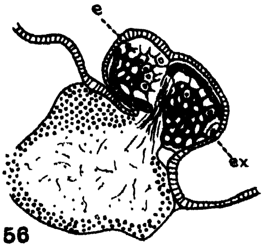

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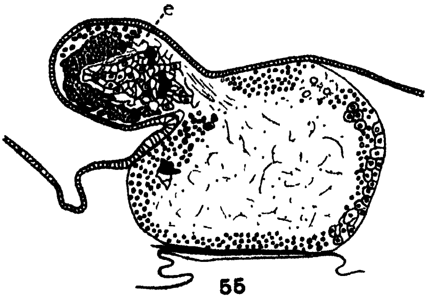

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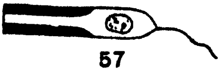

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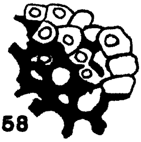

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Fig. 54 *Salpa* (*Ihlea*) *punctata,* aggregated form. A sagittal section through eye and brain. Note the accessory eye in the dorsal part of the brain.

Fig. 55 *Salpa* (*Thetys*) *vagina,* aggregated form. A sagittal section of eye and brain. Note the irregular, degenerate rod cells.

Fig. 56 *Salpa* (*Pegea*) *confederata,* aggregated form. A sagittal section through eye and brain. The optic cell mass at the left (anterior) is all that is left of the dorsal eye. The optic cell mass at the right (posterior) is homologous with the mass of optic cells in the dorsal part of the brain in Salpa punctata (fig. 54).

Fig. 57 A schematic figure of a fully developed rod cell of Salpa.

Fig. 58 A section through a group of irregular, degenerate 'rod cells' of *Salpa* (*Pegea*) *confederata.*

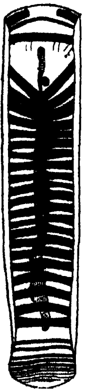
*ex.,* accessory eye; others as in figures 42 to 47.

the right side of the stolon has its left side more cramped, a zooid that has shifted to the left has its right side more cramped. This produces distorting pressure upon one side of each zooid, and we find asymmetrical development of the two sides of the body, the muscles also being affected. As to the eyes, one sees that the left-hand zooids have eyes turned to the right and right-hand zooids have eyes directed to the left.

The habit of living in a cooperative colony has allowed deterioration, and the crowding in the colony has introduced conditions to which asymmetrical development is an adaptation. Does not this suggest how, in the Salpidae, the ancestral character of muscle system and eyes began to be modified? Perfect muscles and perfect eyes are less important in a cooperating communal group, and symmetrical muscles and symmetrical eyes are not so well adapted to the place relations in the chain colony. Adaptive mutations would be encouraged.

But, though these relations suggest how change in ancestral structure may have begun, they do not at all adequately account for the extent to which the changes, once begun, have been carried. The changes, once started, have gone on, apparently of their own momentum, until they have resulted in considerable, yes, in the end, in great degeneration. It is this *momentum of mutation* that is the chief idea in this paper, the habit which mutations so often show of starting a trend. The degenerative changes described in Salpidae seem, so far as they have gone, parallel to numerous instances of animals becoming extinct at the end of a long series of degenerative changes. Disturbance of the developmental controls may lead to results in the end disastrous far beyond the initial changes. Trends once started may also lead to great advantage, favorable characters, insignificant in their early development, thus reaching a condition in which they are of 'selection value.'

It is interesting to see the different effects produced among the Salpidae by introducing alteration in the ancestral control over the muscle-band series. In the family as a whole the trend resulting is toward loss in number of muscles and diminution in their size, but in one subgenus, *Ritteriella,* directly the opposite result appears, the muscle bands increasing in number and width until they form an almost continuous layer in the body wall (figs. 59, 61). In another subgenus, *Thetys,* the number of muscle bands is much increased, but they are short and narrow. Disturbing the ancestral control seems to be uncertain business. It may lead to diverse results.



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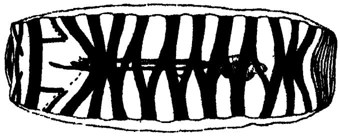

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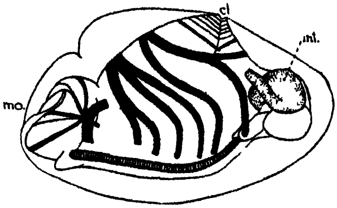

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Fig. 59 *Salpa* (*Ritteriella*) *amboinensis,* solitary form, in dorsal view. Sixteen body-muscle bands may be counted on the middorsal line. Lettering as in figure 29.

Fig. 60 An aggregated zooid of the same species seen from the left side.

Fig. 61 *Salpa* (*Ritteriella*) *picteti,* in dorsal view. Twenty-one body-muscle bands may be counted.

There is a point as to the Salpidae which seems worth emphasis. The changes in each of the three sets of organs, gut, muscles, and eyes, begin in the phyletic series in the aggregated zooids, the last phase of the life-cycle, and later are thrown back upon the solitary Salp, an earlier phase of the life-cycle. The appearance of the new characters in the solitary Salp is an instance of precocious development, like the formation of the heart in vertebrate embryos before the blood-vessel system is well developed, or like the early development and large size of the head in the human foetus. It is often said that such precociously developed organs are those which are of great importance and are of so large size or of so complex character that if they are to be ready to function when needed they must get an early start in development, their development starting earlier and earlier in the ontogeny as, in the course of the phylogeny, their size and complexity increases. According to this generally accepted view, precocious development of organs is a utilitarian phenomenon. The Salpas, however, show precocious development in the solitary animals of characters which in these non-colonial individuals seem not only of no advantage to them, but are even disadvantageous. A chain zooid, one of a colony, can get along with deteriorated muscle bands and eyes, for it shares in the benefits of the muscular activities and light reactions of all the zooids in the chain. The solitary Salp, on the other hand, has to fend for itself without outside aid.

The precocious appearance in the solitary *Salpa* of the deteriorated muscles and eyes characteristic of the aggregated *Salpa* has no reason in utility. On the contrary, there must be some intimate biological cause, in the very nature of the animals, for the tendency of features characteristic of a late phase of the life-cycle to be thrown back upon earlier phases of the life-cycle. In the Salpidae this occurs even to the disadvantage of the solitary *Salpa.*

For the sake of avoiding much circumlocution and constant modifying phrases, we have discussed the idea of trends as if we were endeavoring to establish the hypothesis and prove them to be real, but I am much more interested in developing the idea as a point of view from which to think of the process of evolution. If trends be real, they are highly significant in evolution. Internal factors then become relatively more important in evolution and environmental control is less dominant throughout the successive stages of progress. Of course, in the large, survival or extinction is dependent upon adaptation, but most of the progressing steps in the evolution may be taken without immediate environmental control. Evolution may then be thought of from the standpoint of trends arising in and inhering in the germinal stirp, of their fluctuation in emphasis, increasing or decreasing or perhaps first one and then the other, of their branching, of their shuffling in fertilization like any other characters. Practically all phenomena of phylogeny take on somewhat new aspects from the point of view of trends and their manipulation.

Of especial interest also seems to be the method suggested for studying the reality of trends. The distribution of similar characters in diverse places along the stem and branches of the phylogenetic tree may be such that in explanation one must choose between germinal trends, on the one hand, or multiple occurrences of similar fortuitous mutations, on the other hand, and the latter, to say the least, is highly improbable. These phenomena are most impressive in organisms in which phyletic descent is very clearly indicated. But even in groups with less clearly indicated genetic relationships within the group there often may be two or more characters so distributed in the group as to involve either the presence of trends in the group or multiple fortuitous occurrences of similar characters. The latter, of course, is too improbable for consideration.[8] The course of the phyletic history, so far as subfamilies and genera are concerned, is as clearly indicated in the Opalinidae, I believe, as it is in any group of animals or plants.

The subgeneric groups do not need to be taken into account to give crucial instances of the type of character distribution emphasized, so I have not carried this paper into the minutiae of taxonomic data necessary for adequate discussion of the matter from the standpoint of subgenera. We must be content with the statement that the distribution of characters among the subgeneric groups emphasizes the idea of the presence of trends.

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[8] The main argument in Bergson's "Creative Evolution" is based upon the extreme improbability of repeated fortuitous occurrence of mutations leading continuously in one direction. If these similar modifications are not of fortuitous occurrence, but are rather due to the presence of one or more trends, the force of Bergson's argument is lost, for then we may have for each developing character not a great series of repeated similar independent mutations, but, instead, in the case of each character, a single mutation establishing a trend and thus producing ultimately the developed character.

The taxonomy of the Tunicata is more complex, so it does not seem best to discuss for this group the same sort of crucial distribution of characters. We have therefore merely described for the Tunicates the three trends and the possible origin of two of them.

Of course the objection is sure to be raised that a trend is a mystic, obscure thing of unknown nature and that postulating its existence does not really explain anything, but only makes for vagueness and mystery. What of it? If they seem to be real shall we not take them into account, think about them, test their existence and their behavior by observed phenomena, and even endeavor to find ways of so controlling phenomena as to study trends to still better advantage? Are trends any more mysterious than any other physiological processes, and are the phenomena which indicate them and their behavior in any different class from physiological phenomena in general? We know practically nothing of the intimacies of cell physiology, but we do not, for this reason, abandon physiological studies. Indeed, all physical, including chemical, phenomena are in the same category. All are equally mysterious. Simple explanations of nature, however attractive, are usually not true. If the reality is complex, if it is difficult to study, if methods of attack upon the problems are difficult, what of it? It is up to us to see things as they are, difficulties and all, and to gird our loins for the more laborious study the real conditions present. The idea that a mutation may initiate a trend,[9] if true, is an illuminating and very important one, however little we may know about the origin and real nature of such trends. We may profitably study them and their behavior objectively even if we cannot show, or even imagine, their ultimate nature. If they are real, they and their behavior are significant. The history of evolution may need to be rewritten from the standpoint of trends. Internal factors of evolution may prove of most immediate importance; environmental control being only a final determining or limiting factor in a long series of changes founded upon the nature of the organisms themselves. Few students now hold that all features of structure, habit and physiological behavior in organisms are useful and have been evolved through natural selection because of their utility. Dominant as natural selection is and always must be, it may well be true that it customarily acts only on the end products of certain series out of many long series of changes the preliminary steps of which are indifferent from the standpoint of advantage to the organism.

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[9] If one wishes to avoid in his phraseology any possible flavor of causation, he might say "the idea that a mutation may habitually be followed by a series of more and more emphasized changes in the same direction." I prefer the simpler phrasing, both because it is simpler and because it does give a suggestion of causation.

There are very many phenomena among animals and plants which suggest trends. Senescence and disappearance of species, genera, families, and even of major groups seem to be indicated as often due to trends. Length of life, longer or shorter as the case may be, is a specific character, as truly as is any physiological or structural quality. It is in the stirp. In the species *Homo sapiens*, whether term of life is or is not to any extent a racial quality, it is clearly a heritable quality in families, for the individuals of some families are long-lived, those of others short-lived. We do not know in what rests the control of this matter of persistence of the individual. Whatever the control be, it must be resident in the stirp. Mutation in this germinal quality might be of much importance from the standpoint of survival. If such mutation should initiate a trend, it would be of still greater moment.

The control over length of life of the individual may be related to the capacity for sustaining mitosis without fertilization. It may be related to capacity for sustaining one or another sort of metabolic activity. Structural and functional specialization of at least the more specialized of the soma cells, their fertilization not occurring, seems to interdict their prolonged persistence, though in their germ-cell condition the unspecialized forerunners of these soma cells were able to maintain themselves for an indefinite period, fertilization apparently aiding. Persistence of cell life so long as the cells remain unspecialized is a general quality of germ cells.

But we see in the paleontological record many instances of groups which have shown a progressive series of changes in some direction until they became structurally bizarre and finally became extinct. The gradual over-ornamentation and final extinction of the Trilobites is one such example. The changes made visible by overdevelopment in structure seem to have resulted in unbalanced physiological function or functions and final disaster. The bizarre structural features seem not in themselves hurtful, but they seem rather an indication of physiological unbalance.

The trend to bizarre complication of the growth sutures in the shell among the Cephalopods and that to overdevelopment of the arm skeleton in Brachiopods are two other examples. The increase in size among reptiles in the Cretaceous period and of mammals in Tertiary times seems to have contributed to the extinction of many groups. This is more directly a distortion of physiological habit. In many other groups there appear to have occurred a series of changes involving decreasing vigor or loss of adaptive plasticity, as, for example, among the Bell Toads (Discoglossidae; compare Metcalf, '28). The series of changes in many of these cases may be detectable only in habits or in behavior, and not through the aid of any visible structural index, such as the over-ornamentation of the Trilobites.

The Salpidae, as described, show us how a character may arise as an adaptation of a late phase of the life-cycle, and later this character, of course from its origin a germinal quality, become gradually more and more emphasized in earlier phases of the life-cycle. Mutations giving changes of an adaptive or at least a harmless nature may be thrown back upon earlier stages where they are disadvantageous.

Similarly, loss of vigor affecting at first the old individuals might be thrown back upon earlier stages in the ontogenetic life-history and lead to the decadence or the extermination of the species.

If, at any terminal bud upon the phylogenetic tree, there arise in the stirp a trend toward diminishing vigor, it may pass into all subsequent branches developed from this bud. If among several trends present in any terminal bud there be one leading toward disaster, all the branches later to appear in the growth of this twig may have eventual disaster in their very stirp, and a whole group of species, constituting a subgenus or genus or family, for example, may ultimately come to grief because of this inherent germinal defect. Once recognize the reality of trends and we must include them in our scrutiny of phenomena to see what indications there are of their presence. We must recognize also the possibility of their branching by mutative alteration and look to see if there be indication of this. We must also see if there is indication of their fading out or even of their fluctuating in their effect, from less to greater and to less again and so on. The distinct possibility that most of the evolution that occurs does so by the appearance and disappearance of trends, by their conjunction and disjunction, by their branching, by their waxing and waning or more varied fluctuating, must always be in mind for the student of evolution. I have been so impressed by the irregular occurrence of some character at different places in the taxonomic series, especially among the Opalinidae, that I cannot resist the idea that the tendencies to develop these qualities are in the stirp and that the evolution of the family is largely a matter of the working out of these tendencies, of their conjunction and disjunction, of their temporary suppression and often later reemphasis. Phenomena among the Ophryoscolicidae and the Salpidae strongly reenforce this idea, and many series of conditions among fossil forms seem to point to similar conceptions.

Among those fossil forms including the ancestors of the horse and their relatives there seem to be indications of trends toward increasing emphasis upon the middle digit, toward loss of the outer digits, toward complexity in the teeth, and toward increase in size of the body. The distribution of these qualities along the stem and branches of the phylogenetic tree seems to be such as to involve either trends or independent appearances of similar mutations, the latter, of course, a wholly improbable concept. I have no first-hand familiarity with fossils of the horse tribe and do not care to support the idea of trends with evidence from taxonomic relations in a group of which I have only second-hand knowledge. There seem to be many groups among whose fossil forms not only are trends suggested by increase of characters by increments too slight to be of probable or even conceivable selection value, but among which the occurrence of the several characters is so distributed as to involve either multiple fortuitous mutations of the same sort or, in the case of each such character, a germinal nexus of the sort we have called a trend. But the analyses of such groups from this viewpoint should be made by first-hand students of these groups, and not by an outsider like the present writer. Some of the phyla whose phylogenies are best worth scrutiny from this point of view seem to be among the Ungulates and the Primates, though all phylogenies might well be studied from this viewpoint.

To me trends and their behavior are now the central concept in evolution. But, of course, that adaptation of organisms which we find prevailing is due to natural selection which sets the limits within which trends and their results must keep. It may be that natural selection affects chiefly the later stages in the many series of evolutionary changes which occur because of factors within the organism, and that, of these internal factors of evolution, trends are among the most important.

In all this discussion the term 'orthogenesis' has not been used. It does not seem quite an appropriate and adequate term. Autogenesis or auto-evolution would perhaps better designate the changes that occur from factors within the organism. But the common word trend seems to express even more clearly the idea this paper is written to emphasize. Let me once more say that I am much less interested in establishing the truth of this idea and the value of this method of analysis by reference to any particular phenomena than I am in persuading observers and students to have the concept and the method in mind while ruminating upon the phenomena with which they are dealing.

Many students, especially among paleontologists, have described series of evolutionary changes by successive steps too minute to be each of selection value. In the year 1905, I showed from DeVries' data of mutation in *Oenothera lamarckiana* that the mutation was determinate rather than fortuitous, there being inherent tendency to repeated production of similar mutants. This point was further developed in a paper in the symposium on adaptation at the Cleveland meeting of the American Society of Naturalists in January, 1913 (Metcalf, '13). Similar phenomena of repeated similar mutations are well known in *Drosophila*. It makes little difference from the standpoint of evolution whether we regard *Oenothera* and *Drosophila* as hybrids, for very likely many, or most, or possibly all, plant and animal stocks are hybrid (Lotsy, '22, '25). A dozen years ago, Howard Crawley and I discussed together trends in evolution and such crucial evidence of their existence as is given by the distribution of certain characters along the stems and branches of some phylogenetic trees. His paper upon the Ophryoscolicidae (Crawley, '24) and the present paper discuss only three phylogenies out of very many which might well be analyzed with a view to crucial tests of the presence of trends.

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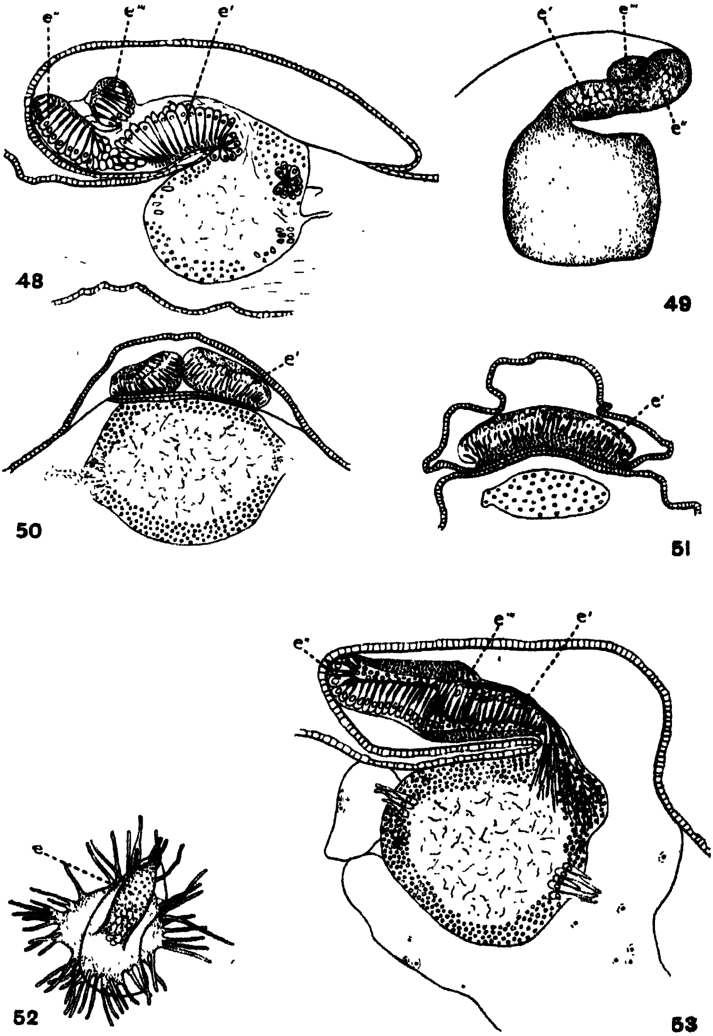
the eye disappear altogether, e.g., the dorsal plug, (figs. 43, 44, 48, 49, 50, 51, 52, 53, 54, 56). At the same time the rod cells lose their regularity of shape and arrangement (figs. 48, 55, 56), their terminal rods are lost and in place we find the whole 'rod cell' wall showing merely irregular glassy thickening (fig. 58, compare fig. 57). Even in the solitary forms of some species the rod cells become degenerate (fig. 47). Somewhat similar changes might be traced for certain accessory eyes present in the brain in the aggregated zooids of different species, but it seems hardly best to include here the descriptions needed to make this clear. The things to emphasize are, first, the progressive degeneration of the eyes and, secondly, that in these phylogenetic changes the aggregated zooids lead and the solitary Salps follow.

The Salpas show thus three trends: 1) toward coiling of the gut; 2) toward simplification and irregular arrangement of the body-muscle series, and, 3) toward degeneration of the eyes.

We have thus far noted the presence of five trends in the family Opalinidae, of two in the Ophryoscolecidae, and of three in the Salpidae. In the case of the Salpidae we find some indication of how two of these trends may have started. I see no clear suggestion as to the origin of the trend toward compact coiling of the gut, except that in the phylogeny of the Salpidae the exhalent aperture has been drawn backward from an originally more dorsal position, such as is seen in the ascidians, and that it might be advantageous for the anus also to move back so as to be near the exhalent siphon. But the degeneration of the eyes and of the muscle system may well have been permitted by the habit of the aggregated individuals of living in a chain. The solitary Salps have to fend each for itself. But the aggregated zooids live most of their lives as members of a more or less cooperative community, the 'chain.' The young chain individuals are carried by the solitary Salp and have little need of muscles or eyes of their own. Only after they reach their full development, except for size, are they freed from the chain, and then a number of



Figures 48 to 53

individuals usually break away together forming quite a long colony, and they continue bound together, though no longer attached to the solitary Salp. Thus, during the whole period of their development and usually until sexual reproduction is accomplished, they are members of a cooperative colony. Because of the mutual aid between individual zooids, there is less need for each zooid to have a full and perfectly developed series of muscles or perfect eyes. Natural selection does not operate so keenly upon these cooperating zooids in respect to their muscles and eyes.

But this is not all that is involved. Life in a chain group does not merely permit departure from the ancestral arrangement and structure of muscles and eyes; it goes further and promotes changes in these organs. A word about the arrangement of the zooids in the chain is necessary to make the matter clear. In the *Salpa* chain, as indeed throughout the Tunicata, bud-formed individuals arise with the dorsal surface toward the parent and the ventral surface away from the parent; belly to back is the position. On the *Salpa* chain many buds are serially formed by constriction of the stolon, the oldest bud at the tip of the stolon and the successively younger buds forming in series toward the base of the stolon. In the young stolon the bud zooids lie in a linear series belly to back. But, as the zooids increase in size and crowd one another, they change their relative positions, becoming in most species apparently a double row of zooids, though not really so in fundamental relations. This is brought about by the zooids shifting alternately to the right and to the left, no.

**Fig. 48** A parasagittal section of the eye and brain of *Salpa* (*Cyclosalpa*) *pinnata*, aggregated form. In this figure the anterior end is at the left, the reverse of figure 44.

**Fig. 49** The eye and brain of *Salpa* (*Cyclosalpa*) *affinis*, aggregated form, seen from the right side.

**Fig. 50** *Salpa* (*Cyclosalpa*) *pinnata*, aggregated form. A transverse section of the posterior portion of the eye (the brain also showing).

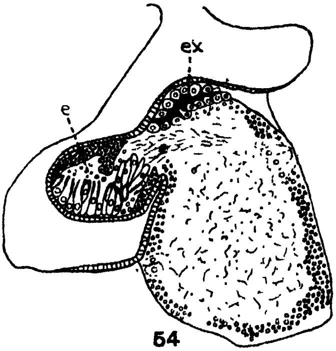
**Fig. 51** A similar section from *Salpa* (*Cyclosalpa*) *affinis*.

**Fig. 52** *Salpa* (*Cyclosalpa*) *virgula*, aggregated form. The eye and brain.

**Fig. 53** A sagittal section of the same.

Lettering as in figures 42 to 47.

1 to the right, no. 2 to the left, no. 3 to the right, and so on. The result is a double row of diagonally placed individuals (compare Brooks, '93) in which one side of each zooid is more cramped than the other side. The zooid that has shifted to



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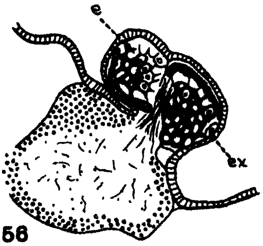

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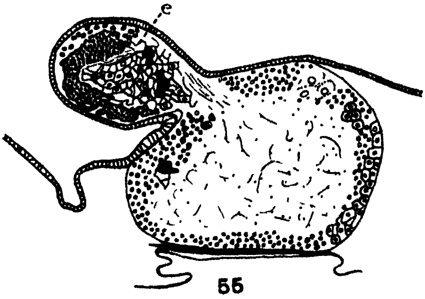

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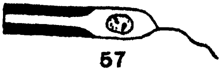

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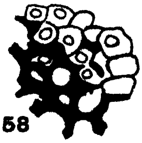

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Fig. 54 *Salpa* (*Ihlea*) *punctata*, aggregated form. A sagittal section through eye and brain. Note the accessory eye in the dorsal part of the brain.

Fig. 55 *Salpa* (*Thetys*) *vagina*, aggregated form. A sagittal section of eye and brain. Note the irregular, degenerate rod cells.

Fig. 56 *Salpa* (*Pegea*) *confederata*, aggregated form. A sagittal section through eye and brain. The optic cell mass at the left (anterior) is all that is left of the dorsal eye. The optic cell mass at the right (posterior) is homologous with the mass of optic cells in the dorsal part of the brain in Salpa punctata (fig. 54).

Fig. 57 A schematic figure of a fully developed rod cell of Salpa.

Fig. 58 A section through a group of irregular, degenerate 'rod cells' of *Salpa* (*Pegea*) *confederata*.

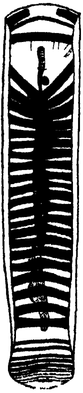
*ex.*, accessory eye; others as in figures 42 to 47.

the right side of the stolon has its left side more cramped, a zooid that has shifted to the left has its right side more cramped. This produces distorting pressure upon one side of each zooid, and we find asymmetrical development of the two sides of the body, the muscles also being affected. As to the eyes, one sees that the left-hand zooids have eyes turned to the right and right-hand zooids have eyes directed to the left.

The habit of living in a cooperative colony has allowed deterioration, and the crowding in the colony has introduced conditions to which asymmetrical development is an adaptation. Does not this suggest how, in the Salpidae, the ancestral character of muscle system and eyes began to be modified? Perfect muscles and perfect eyes are less important in a cooperating communal group, and symmetrical muscles and symmetrical eyes are not so well adapted to the place relations in the chain colony. Adaptive mutations would be encouraged.

But, though these relations suggest how change in ancestral structure may have begun, they do not at all adequately account for the extent to which the changes, once begun, have been carried. The changes, once started, have gone on, apparently of their own momentum, until they have resulted in considerable, yes, in the end, in great degeneration. It is this *momentum of mutation* that is the chief idea in this paper, the habit which mutations so often show of starting a trend. The degenerative changes described in Salpidae seem, so far as they have gone, parallel to numerous instances of animals becoming extinct at the end of a long series of degenerative changes. Disturbance of the developmental controls may lead to results in the end disastrous far beyond the initial changes. Trends once started may also lead to great advantage, favorable characters, insignificant in their early development, thus reaching a condition in which they are of 'selection value.'

It is interesting to see the different effects produced among the Salpidae by introducing alteration in the ancestral control over the muscle-band series. In the family as a whole the trend resulting is toward loss in number of muscles and diminution in their size, but in one subgenus, *Ritteriella,* directly the opposite result appears, the muscle bands increasing in number and width until they form an almost continuous layer in the body wall (figs. 59, 61). In another subgenus, *Thetys*, the number of muscle bands is much increased, but they are short and narrow. Disturbing the ancestral control seems to be uncertain business. It may lead to diverse results.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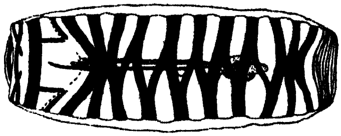
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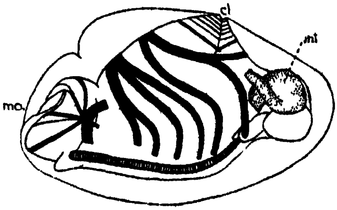

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Fig. 59 *Salpa* (*Ritteriella*) *amboinensis,* solitary form, in dorsal view. Sixteen body muscle bands may be counted on the middorsal line. Lettering as in figure 29.

Fig. 60 An aggregated zooid of the same species seen from the left side.

Fig. 61 *Salpa* (*Ritteriella*) *picteti,* in dorsal view. Twenty-one body-muscle bands may be counted.

There is a point as to the Salpidae which seems worth emphasis. The changes in each of the three sets of organs, gut, muscles, and eyes, begin in the phyletic series in the aggregated zooids, the last phase of the life-cycle, and later are thrown back upon the solitary Salp, an earlier phase of the life-cycle. The appearance of the new characters in the solitary Salp is an instance of precocious development, like the formation of the heart in vertebrate embryos before the blood-vessel system is well developed, or like the early development and large size of the head in the human foetus. It is often said that such precociously developed organs are those which are of great importance and are of so large size or of so complex character that if they are to be ready to function when needed they must get an early start in development, their development starting earlier and earlier in the ontogeny as, in the course of the phylogeny, their size and complexity increases. According to this generally accepted view, precocious development of organs is a utilitarian phenomenon. The Salpas, however, show precocious development in the solitary animals of characters which in these non-colonial individuals seem not only of no advantage to them, but are even disadvantageous. A chain zooid, one of a colony, can get along with deteriorated muscle bands and eyes, for it shares in the benefits of the muscular activities and light reactions of all the zooids in the chain. The solitary Salp, on the other hand, has to fend for itself without outside aid.

The precocious appearance in the solitary *Salpa* of the deteriorated muscles and eyes characteristic of the aggregated *Salpa* has no reason in utility. On the contrary, there must be some intimate biological cause, in the very nature of the animals, for the tendency of features characteristic of a late phase of the life-cycle to be thrown back upon earlier phases of the life-cycle. In the Salpidae this occurs even to the disadvantage of the solitary *Salpa.*

For the sake of avoiding much circumlocution and constant modifying phrases, we have discussed the idea of trends as if we were endeavoring to establish the hypothesis and prove them to be real, but I am much more interested in developing the idea as a point of view from which to think of the process of evolution. If trends be real, they are highly significant in evolution. Internal factors then become relatively more important in evolution and environmental control is less dominant throughout the successive stages of progress. Of course, in the large, survival or extinction is dependent upon adaptation, but most of the progressing steps in the evolution may be taken without immediate environmental control. Evolution may then be thought of from the standpoint of trends arising in and inhering in the germinal stirp, of their fluctuation in emphasis, increasing or decreasing or perhaps first one and then the other, of their branching, of their shuffling in fertilization like any other characters. Practically all phenomena of phylogeny take on somewhat new aspects from the point of view of trends and their manipulation.

Of especial interest also seems to be the method suggested for studying the reality of trends. The distribution of similar characters in diverse places along the stem and branches of the phylogenetic tree may be such that in explanation one must choose between germinal trends, on the one hand, or multiple occurrences of similar fortuitous mutations, on the other hand, and the latter, to say the least, is highly improbable. These phenomena are most impressive in organisms in which phyletic descent is very clearly indicated. But even in groups with less clearly indicated genetic relationships within the group there often may be two or more characters so distributed in the group as to involve either the presence of trends in the group or multiple fortuitous occurrences of similar characters. The latter, of course, is too improbable for consideration.[8] The course of the phyletic history, so far as subfamilies and genera are concerned, is as clearly indicated in the Opalinidae, I believe, as it is in any group of animals or plants.

The subgeneric groups do not need to be taken into account to give crucial instances of the type of character distribution emphasized, so I have not carried this paper into the minutiae of taxonomic data necessary for adequate discussion of the matter from the standpoint of subgenera. We must be content with the statement that the distribution of characters among the subgeneric groups emphasizes the idea of the presence of trends.

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[8] The main argument in Bergson's "Creative Evolution" is based upon the extreme improbability of repeated fortuitous occurrence of mutations leading continuously in one direction. If these similar modifications are not of fortuitous occurrence, but are rather due to the presence of one or more trends, the force of Bergson's argument is lost, for then we may have for each developing character not a great series of repeated similar independent mutations, but, instead, in the case of each character, a single mutation establishing a trend and thus producing ultimately the developed character.

The taxonomy of the Tunicata is more complex, so it does not seem best to discuss for this group the same sort of crucial distribution of characters. We have therefore merely described for the Tunicates the three trends and the possible origin of two of them.

Of course the objection is sure to be raised that a trend is a mystic, obscure thing of unknown nature and that postulating its existence does not really explain anything, but only makes for vagueness and mystery. What of it? If they seem to be real shall we not take them into account, think about them, test their existence and their behavior by observed phenomena, and even endeavor to find ways of so controlling phenomena as to study trends to still better advantage? Are trends any more mysterious than any other physiological processes, and are the phenomena which indicate them and their behavior in any different class from physiological phenomena in general? We know practically nothing of the intimacies of cell physiology, but we do not, for this reason, abandon physiological studies. Indeed, all physical, including chemical, phenomena are in the same category. All are equally mysterious. Simple explanations of nature, however attractive, are usually not true. If the reality is complex, if it is difficult to study, if methods of attack upon the problems are difficult, what of it? It is up to us to see things as they are, difficulties and all, and to gird our loins for the more laborious study the real conditions present. The idea that a mutation may initiate a trend,[9] if true, is an illuminating and very important one, however little we may know about the origin and real nature of such trends. We may profitably study them and their behavior objectively even if we cannot show, or even imagine, their ultimate nature. If they are real, they and their behavior are significant. The history of evolution may need to be rewritten from the standpoint of trends. Internal factors of evolution may prove of most immediate importance; environmental control being only a final determining or limiting factor in a long series of changes founded upon the nature of the organisms themselves. Few students now hold that all features of structure, habit and physiological behavior in organisms are useful and have been evolved through natural selection because of their utility. Dominant as natural selection is and always must be, it may well be true that it customarily acts only on the end products of certain series out of many long series of changes the preliminary steps of which are indifferent from the standpoint of advantage to the organism.

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[9] If one wishes to avoid in his phraseology any possible flavor of causation, he might say "the idea that a mutation may habitually be followed by a series of more and more emphasized changes in the same direction." I prefer the simpler phrasing, both because it is simpler and because it does give a suggestion of causation.

There are very many phenomena among animals and plants which suggest trends. Senescence and disappearance of species, genera, families, and even of major groups seem to be indicated as often due to trends. Length of life, longer or shorter as the case may be, is a specific character, as truly as is any physiological or structural quality. It is in the stirp. In the species *Homo sapiens,* whether term of life is or is not to any extent a racial quality, it is clearly a heritable quality in families, for the individuals of some families are long-lived, those of others short-lived. We do not know in what rests the control of this matter of persistence of the individual. Whatever the control be, it must be resident in the stirp. Mutation in this germinal quality might be of much importance from the standpoint of survival. If such mutation should initiate a trend, it would be of still greater moment.

The control over length of life of the individual may be related to the capacity for sustaining mitosis without fertilization. It may be related to capacity for sustaining one or another sort of metabolic activity. Structural and functional specialization of at least the more specialized of the soma cells, their fertilization not occurring, seems to interdict their prolonged persistence, though in their germ-cell condition the unspecialized forerunners of these soma cells were able to maintain themselves for an indefinite period, fertilization apparently aiding. Persistence of cell life so long as the cells remain unspecialized is a general quality of germ cells.

But we see in the paleontological record many instances of groups which have shown a progressive series of changes in some direction until they became structurally bizarre and finally became extinct. The gradual over-ornamentation and final extinction of the Trilobites is one such example. The changes made visible by overdevelopment in structure seem to have resulted in unbalanced physiological function or functions and final disaster. The bizarre structural features seem not in themselves hurtful, but they seem rather an indication of physiological unbalance.

The trend to bizarre complication of the growth sutures in the shell among the Cephalopods and that to overdevelopment of the arm skeleton in Brachiopods are two other examples. The increase in size among reptiles in the Cretaceous period and of mammals in Tertiary times seems to have contributed to the extinction of many groups. This is more directly a distortion of physiological habit. In many other groups there appear to have occurred a series of changes involving decreasing vigor or loss of adaptive plasticity, as, for example, among the Bell Toads (Discoglossidae; compare Metcalf, '28). The series of changes in many of these cases may be detectable only in habits or in behavior, and not through the aid of any visible structural index, such as the over-ornamentation of the Trilobites.

The Salpidae, as described, show us how a character may arise as an adaptation of a late phase of the life-cycle, and later this character, of course from its origin a germinal quality, become gradually more and more emphasized in earlier phases of the life-cycle. Mutations giving changes of an adaptive or at least a harmless nature may be thrown back upon earlier stages where they are disadvantageous.

Similarly, loss of vigor affecting at first the old individuals might be thrown back upon earlier stages in the ontogenetic life-history and lead to the decadence or the extermination of the species.

If, at any terminal bud upon the phylogenetic tree, there arise in the stirp a trend toward diminishing vigor, it may pass into all subsequent branches developed from this bud. If among several trends present in any terminal bud there be one leading toward disaster, all the branches later to appear in the growth of this twig may have eventual disaster in their very stirp, and a whole group of species, constituting a subgenus or genus or family, for example, may ultimately come to grief because of this inherent germinal defect. Once recognize the reality of trends and we must include them in our scrutiny of phenomena to see what indications there are of their presence. We must recognize also the possibility of their branching by mutative alteration and look to see if there be indication of this. We must also see if there is indication of their fading out or even of their fluctuating in their effect, from less to greater and to less again and so on. The distinct possibility that most of the evolution that occurs does so by the appearance and disappearance of trends, by their conjunction and disjunction, by their branching, by their waxing and waning or more varied fluctuating, must always be in mind for the student of evolution. I have been so impressed by the irregular occurrence of some character at different places in the taxonomic series, especially among the Opalinidae, that I cannot resist the idea that the tendencies to develop these qualities are in the stirp and that the evolution of the family is largely a matter of the working out of these tendencies, of their conjunction and disjunction, of their temporary suppression and often later reemphasis. Phenomena among the Ophryoscolicidae and the Salpidae strongly reenforce this idea, and many series of conditions among fossil forms seem to point to similar conceptions.

Among those fossil forms including the ancestors of the horse and their relatives there seem to be indications of trends toward increasing emphasis upon the middle digit, toward loss of the outer digits, toward complexity in the teeth, and toward increase in size of the body. The distribution of these qualities along the stem and branches of the phylogenetic tree seems to be such as to involve either trends or independent appearances of similar mutations, the latter, of course, a wholly improbable concept. I have no first-hand familiarity with fossils of the horse tribe and do not care to support the idea of trends with evidence from taxonomic relations in a group of which I have only second-hand knowledge. There seem to be many groups among whose fossil forms not only are trends suggested by increase of characters by increments too slight to be of probable or even conceivable selection value, but among which the occurrence of the several characters is so distributed as to involve either multiple fortuitous mutations of the same sort or, in the case of each such character, a germinal nexus of the sort we have called a trend. But the analyses of such groups from this viewpoint should be made by first-hand students of these groups, and not by an outsider like the present writer. Some of the phyla whose phylogenies are best worth scrutiny from this point of view seem to be among the Ungulates and the Primates, though all phylogenies might well be studied from this viewpoint.

To me trends and their behavior are now the central concept in evolution. But, of course, that adaptation of organisms which we find prevailing is due to natural selection which sets the limits within which trends and their results must keep. It may be that natural selection affects chiefly the later stages in the many series of evolutionary changes which occur because of factors within the organism, and that, of these internal factors of evolution, trends are among the most important.

In all this discussion the term 'orthogenesis' has not been used. It does not seem quite an appropriate and adequate term. Autogenesis or auto-evolution would perhaps better designate the changes that occur from factors within the organism. But the common word trend seems to express even more clearly the idea this paper is written to emphasize. Let me once more say that I am much less interested in establishing the truth of this idea and the value of this method of analysis by reference to any particular phenomena than I am in persuading observers and students to have the concept and the method in mind while ruminating upon the phenomena with which they are dealing.

Many students, especially among paleontologists, have described series of evolutionary changes by successive steps too minute to be each of selection value. In the year 1905, I showed from DeVries' data of mutation in *Oenothera lamarckiana* that the mutation was determinate rather than fortuitous, there being inherent tendency to repeated production of similar mutants. This point was further developed in a paper in the symposium on adaptation at the Cleveland meeting of the American Society of Naturalists in January, 1913 (Metcalf, '13). Similar phenomena of repeated similar mutations are well known in *Drosophila*. It makes little difference from the standpoint of evolution whether we regard *Oenothera* and *Drosophila* as hybrids, for very likely many, or most, or possibly all, plant and animal stocks are hybrid (Lotsy, '22, '25). A dozen years ago, Howard Crawley and I discussed together trends in evolution and such crucial evidence of their existence as is given by the distribution of certain characters along the stems and branches of some phylogenetic trees. His paper upon the Ophryoscolicidae (Crawley, '24) and the present paper discuss only three phylogenies out of very many which might well be analyzed with a view to crucial tests of the presence of trends.

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# A STUDY OF THE CHROMOSOMES IN MEIOSIS, FERTILIZATION, AND CLEAVAGE IN THE GRASSHOPPER EGG (ORTHOPTERA)[1]

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NINE PLATES (SIXTY-SIX FIGURES)

AUTHOR'S ABSTRACT

A technique is developed whereby the large heavily yolk-laden grasshopper egg may be sectioned for cytological study. Eggs of Chortophaga viridifasciata and Circotettix verruculatus were examined.

In C. viridifasciata the structure of the late ovarian nucleus and the chromosomes in meiosis, fertilization, and early cleavage are described. In the first maturation twelve rod-shaped tetrads are found. Near the caudal end of the egg a polar body is given off, and in the second maturation division there are twelve dyads. At fertilization, twelve separated vesicles of the female pronucleus are seen scattered about a male pronucleus in which the chromosomes are in prophase.

In early cleavage the chromosome numbers in the metaphase are found to differentiate the male- and female-producing eggs. In the former there are twenty-three and in the latter twenty-four chromosomes. A vesicular condition in which the chromosomes retain their boundaries in interkinesis is indicative of chromosome individuality.

A comparison of the first and second maturation metaphase chromosomes of the oocyte with those of the spermatocyte shows a similar compact group on the spindle, a likeness in size seriation, and a similarity of form. They differ in that in the spermatocyte complex there are eleven tetrads and one dyad. This latter, the unpaired sex chromosome, falls among the large chromosomes.

A comparison of the chromosome complex of Chortophaga viridifasciata with that of Circotettix verruculatus indicates constancy of generic differences in form, size, number, and behavior of the chromosomes.

## CONTENTS

Introduction ... 48
  Statement of the problem ... 48
  Acknowledgment ... 49
  Material studied ... 50
Technique ... 50
Observations ... 53
  1. Oogenesis ... 53
    Species studied ... 53
    Ovarian structure ... 53
    Process of oviposition and structure of the egg pod ... 54
    Periods at which meiosis, fertilization, and the formation of the blastoderm and of the germ band occur ... 54
    Description of the egg in the follicle ... 55
    Description of the egg after oviposition ... 56

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[1] A thesis in zoölogy presented to the faculty of the graduate school of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

    The maturation process ... 57
    Description of maturation as found in Chortophaga viridifasciata. 57
    First maturation ... 57
    Second maturation and polar bodies ... 58
  2. Fertilization ... 59
    The sperm in the egg ... 59
    The male and female pronuclei ... 60
  3. Cleavage ... 60
    Early cleavage nuclei ... 60
    Chromosome conditions in early cleavage ... 61
      Metaphase ... 61
      Anaphase ... 61
      Telophase ... 61
      Prophase ... 62
      Chromosome numbers ... 62
  4. Comparison of the metaphase chromosomes of the oocyte with those of the spermatocyte in Chortophaga viridifasciata ... 62
  5. Comparison of the metaphase chromosomes of the oocyte in Circotettix verruculatus with those in the spermatocyte in the same species and with those in the oocyte of Chortophaga viridifasciata. 64
Reviews and discussions ... 65
  Oogenesis in insects ... 65
    Review of papers dealing with orthopteran oogenesis ... 66
    Discussion of orthopteran oogenesis ... 68
    Recent papers on oogenesis in other orders of insects ... 69
    Chromosomes in spermatogenesis ... 73
    Significance of chromosome vesicles ... 72
Summary ... 75
Bibliography ... 76

## INTRODUCTION

### *Statement of the problem*

The constancy of numbers, forms, and behavior of the chromosomes in the complex of many species, genera, and higher groups of Acrididae has been demonstrated by Doctor McClung and his students. It appears that in many of the groups of short-horned grasshoppers the somatic cells of the male contain twenty-three chromosomes, while those of the female contain twenty-four.

As has been reported by Doctor McClung ('05, '14, '17) for most acridians, the spermatogonia contain an odd number of chromosomes, namely, twenty-three, which reduce to twelve in the first spermatocyte, eleven tetrads and one dyad. A dimorphism results when in the first maturation the unpaired accessory chromosome fails to divide. Thus, of the four cells produced by the two divisions, two contain eleven chromosomes and two contain twelve, i.e., one-half the spermatids contain the accessory and one-half lack it.

The observed facts that there are two essentially different forms of spermatozoa in acridians correlated with the tendency of the species to perpetuate in two sexes of approximately equal numbers, suggested to Doctor McClung in 1902 that the accessory chromosome functions as a sex determinant. This theory assumed all ova to be of a single type, and the present work verifies that assumption. In the follicle cells of the ovary twenty-four chromosomes are found demonstrating the female somatic complex. It is shown later that twelve tetrads are found in the first oocyte and all ootids contain twelve chromosomes at the end of meiosis. Accordingly, fertilization produces two types of individuals, differentiated primarily by their chromosome complex, the male containing twenty-three and the female twenty-four chromosomes in the somatic cells.

Since 1900, Doctor McClung and his students have been studying orthopteran spermatogenesis. Due to technical difficulties, the chromosome conditions in oogenesis have remained unknown, and this has left without observational basis the assumption of a monomorphic condition of the eggs. The present contribution is the first to supply this lack and to complete the picture of orthopteran gametogenesis upon which the chromosome theory of sex is based.

The observations in this paper are limited to a study of the late ovarian stages of the ovum and to the chromosome conditions as found in the egg after oviposition.

## ACKNOWLEDGMENT

This problem was suggested by Dr. C. E. McClung, to whom the author wishes to express her sincere appreciation for his extreme interest, encouragement, and assistance in the study of general chromosome conditions. The author also

thanks Dr. E. Eleanor Carothers, who has given valuable assistance in technique and in the preparation of the photomicrographs, and also helped in the study of the Circotettix preparations.

## MATERIAL STUDIED

The material studied was taken mainly from Chortophaga viridifasciata. The grasshoppers were collected in Delaware County, Pennsylvania. In this species the animals hatch out in the latter part of the summer and hibernate as nymphs of the third instar, normally maturing about May in this locality. However, when collected in the fall and kept indoors, they continue to grow and the females lay eggs by January. Eggs from animals raised in this manner, as well as from animals collected after they had become adult, were studied.

The eggs from two pods of a Circotettix verruculatus stock upon which Doctor Carothers was carrying out genetical experiments were also studied.

## TECHNIQUE

*Fixation.* The preparation of the newly laid grasshopper egg occasioned considerable trouble. Due to the large amount of yolk and thick chitinous chorion present, it is difficult for fixatives to penetrate and give good fixation of the egg nucleus and early embryonic cells. Several fixatives were tried and, as most favorable results were obtained with Carnoy-Lebrum, it was subsequently used. This fixative consists of equal parts of chloroform, absolute alcohol, and glacial acetic acid saturated with mercuric chloride. It penetrates the chorion and yolk, giving good fixation of cells scattered about in the yolk as well as those on the periphery. The chromosome fixation is good, although it is generally considered to give poor cytoplasmic fixation. The yolk sections better in celloidin when this solution is used.

*Procedure for fixing and preserving.* Before fixing, the eggs are removed from the pod, the sand and secretion clinging to the chorion are cleaned off. (It is easier to clean the eggs under water, especially if they have been laid some time, as the secretion about them has then had time to harden.)

The eggs are then placed in Carnoy-Lebrum (at room temperature) for ten minutes, allowing about 1 cc. of fixing fluid per egg. After about five minutes, the chorion of each egg is punctured with a fine steel needle. (The puncture should be made in a lateral position, thus avoiding the critical region about the caudal end. The yolk should be sufficiently hardened so that it does not exude from the puncture.)

From the fixing fluid they are transferred immediately to weakly iodized 70 per cent alcohol for about twenty-four hours, or until the solution is no longer decolorized. Several changes may be necessary. (This washing removes the excess of mercuric chloride.) The eggs are then transferred to 70 per cent alcohol, where they can be left indefinitely.

*Sectioning.* In sectioning, both celloidin and paraffin methods were tried. The celloidin method was found to be the only feasible method, and was used entirely for the preparations studied. The paraffin method[2] was not satisfactory. on account of the difficulty in cutting the brittle yolk, and because heat causes clumping of the chromosomes and shrinkage of the large cells.

*Procedure for the celloidin method.* In dehydrating, preparatory to celloidin infiltration, the eggs, fixed according to the above directions and preserved in 70 per cent alcohol, are transferred to 83 per cent alcohol for two hours; 95 per cent alcohol, three changes, for twenty-four hours; absolute alcohol, two changes, two to four hours; equal parts absolute alcohol and ether, one to two hours. They are then placed in thick celloidin for two to four weeks. The chorion should be punctured in the lateral region under the celloidin. (The corked vial is dipped in melted paraffin to make it air-tight and insure against evaporation.)

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[2] The most successful procedure for the paraffin method is as follows: The eggs are fixed and preserved according to the preceding directions. Dehydration in 83 per cent alcohol for two hours, in two changes of 95 per cent alcohol for one hour each, precedes clearing in turpentine, two changes, one hour each. The turpentine is removed by chloroform, two changes, five minutes each. The egg is then placed in melted soft paraffin (55° to 58°) for one hour, followed by hard paraffin (58° to 62°) for one hour.

When infiltration is complete, the cork is loosened while the celloidin gradually thickens. After about a week, the cork may be removed and then the celloidin in which the infiltrated eggs are embedded should be firm enough to be removed from the vial. The excess celloidin is trimmed off and the remainder containing the eggs placed in chloroform vapor (about an hour) to harden and then into 70 per cent alcohol indefinitely. Or the eggs may be trimmed separately and blocked at this time. Celloidin containing infiltrated tissue should never be allowed to become dry.

Each egg is blocked separately. Longitudinal or oblique sections are more convenient to cut than cross-sections. The infiltrated egg, with a small amount of celloidin surrounding it, is placed successively in 95 per cent alcohol, absolute alcohol, and in a mixture of equal parts of absolute alcohol and ether, sufficiently long (about one minute in each) to dehydrate the outer surface of the celloidin. Meanwhile a fairly thick solution of celloidin is poured over the surface of a fiberoid block which can be fastened in the microtome. The egg is then thrust into the celloidin on the block. After the fresh celloidin has begun to harden, or in about ten minutes, the whole is placed in chloroform vapor until the celloidin is very firm, but not dry. The blocked egg must then be kept in 70 per cent alcohol for at least twenty-four hours, after which it may be sectioned at any time.

In making serial sections with celloidin, the eggs are cut at 12 μ with a Minot precision microtome. As the sections are cut, each one is pulled to the back of the knife with a sable brush. Here they are arranged serially in parallel rows. It is necessary to keep the sections and the blocked egg constantly wet with 60 per cent alcohol.

When the entire egg is sectioned, the sections are transferred serially to a chemically clean slip. This is accomplished by first pulling them on to a safety-razor blade with a dissecting needle, and then on to the slip. The length of the rows should be made to correspond to the length of the cover-slip used, and the same number of sections should be in each row. (The author used 22 × 40 mm. no. 0 cover-slips.)

After each slip is filled (an egg about fills two slips), the moist sections are blotted with filter-paper to press out all excess alcohol. A thin solution of celloidin is immediately flooded over the sections with a pipette, and this coating is quickly rinsed with a mixture of equal parts absolute alcohol and ether to remove as much of the celloidin as possible, but still leave sufficient to prevent the sections from becoming displaced. The slip on which the sections are mounted is then placed in 70 per cent alcohol until it is convenient to stain. The sectioned egg, thus mounted, was mordanted in 4 per cent iron alum, stained in a ½ per cent solution of Heidenhain's haematoxylin, and mounted in damar.

## OBSERVATIONS

### 1. *Oogenesis*

*Species studied.* The observations are based mainly upon Chortophaga viridifasciata. A few eggs of Circotettix verruculatus were also studied. Both these species belong to the subfamily Oedipodinae of the orthopteran family Acrididae. Spermatogenesis in C. viridifasciata was reported by McClung in 1905 and Davis in 1908, and in C. verruculatus by Carothers in 1921.

*Ovarian structure.* The two ovaries of the grasshopper consist of a number of tubes lying in the abdominal cavity dorsal to the alimentary canal. Each set of tubes opens into an oviduct situated laterally. The two oviducts at their caudal ends open into a common duct, the vagina, which in turn opens through the ovipositors to the exterior. The ovarian tubes are of the holoistic type, i.e., a type in which each germ cell is potentially functional, while the follicle cells surrounding each ovum supply the nutriment. The ova are found in successive stages of growth in the ovariole. The largest and oldest eggs are near the oviduct.

*Process of oviposition and structure of the egg pod.* In the process of laying eggs in the laboratory, the female grasshopper drills a hole with her ovipositors in the firmly packed sand which has been placed about 4 inches deep in the cage or in a wide-mouth jar. Sometimes she lays the eggs against the inside of the glass container and the process may then be observed. The transparent integument between the sclerites is as wide as the sclerites when the abdomen is fully extended, and the movement of the internal organs may be superficially observed. When she begins laying she throws out a white secretion, churning it with her ovipositors, then after a moment of rest deposits an egg. She repeats these actions until the process is finished, which usually requires about three-quarters of an hour. The abdomen is gradually shortened as laying proceeds, and when almost completed the integument has disappeared under the sclerites. The hole into which the abdomen was inserted is filled almost to the surface with excess secretion and finally covered over with sand by the hind legs. The secretion hardens and forms a protective mass around the eggs. Usually, in C. viridifasciata, there are twelve to fifteen eggs to a pod, but the number may vary from seven to twenty. They occupy one-fourth to three-fourths of the pod, depending both upon the number of eggs and the distance from the surface that the pod was begun. The eggs are arranged in two parallel rows. Each row is composed of eggs parallel to each other and placed obliquely in the long axis of the pod.

*Periods at which meiosis, fertilization, and formation of the blastoderm and of the germ band occur.* The maturation divisions are initiated at the time the egg is laid, but are perhaps affected by entrance of the sperm. Apparently, four to six hours are required for completion of the first and second maturation divisions. By the end of the first day, the pronuclei have united and cleavage has begun. In the second and third days, a few very large cells are to be found scattered in the yolk. At seven or eight days, a migration to the periphery of the caudal third of the egg is well advanced and there, by repeated division of the cells, the blastoderm is formed. At about three weeks, the germ band has invaginated and is situated in the caudal end of the egg. The serosa completely surrounds the egg and the amnion is separated from the serosa by the yolk.

The entire history of the chromosomes throughout all the stages of maturation and early cleavage has not been observed, due to insufficiency of material. One mature egg, of which there are about ninety sections when cut at 12 μ, can show only one stage in the whole process. Hundreds of eggs were sectioned, but repetition of the same stages are numerous, even though the eggs were fixed at varying intervals after laying.

*Description of the egg in the follicle.* Accompanying the striking increase in volume of the egg during the ovarian growth period, there is a change in the nuclear and cytoplasmic structures. A process of diffusion goes on from a condensed state of the chromosomes as found in the early stages of oogenesis (studied in ovaries in which some of the eggs are completely matured) to a condition in the mature germinal vesicle in which no definite organization of the chromosomes is presented. Figure 1 represents a section through the distal end of an ovariole. The egg nucleus *a*, of an oocyte, is one in which the chromosomes appear as thick and twisted threads, each discrete and separate. The threads appear to be lengthening. In the oocyte nucleus *b* the chromosomes are more diffuse and a longitudinal split is visible. Figure 9 is a polar view of a metaphase plate of a dividing follicle cell. There are twenty-four chromosomes, the somatic number in the female of C. viridifasciata.

An oocyte nucleus which has attained its maximum size is shown in figure 5. Diffuse chromatic threads are typically grouped together and lie near the periphery. Small particles of chromatin are seen throughout the nuclear network. Deeply staining spherical bodies are scattered about the nucleus. No distinct cytoplasmic region surrounds the germinal vesicle. The yolk at this stage is finely granular.

Situated in the caudal end of the egg in the late ovarian stage, the germinal vesicle presents itself as a spheroidal nucleus about 25 or 30 μ in diameter (fig. 2). It is surrounded by a thin cytoplasmic layer, from the periphery of which extend irregular short amoeboid processes into the yolk. The chromatic structures of the nucleus are very indefinite and diffusely staining. Deeply staining spherical bodies are numerous, in some instances appearing to be a part of the chromatic network, in others, quite separate. They are also found scattered about in the completely formed yolk. At this stage the follicular cells (fig. 6—magnification one-fourth that of the germinal vesicle, fig. 2) appear to be functioning actively in secreting material for the yolk, as evidenced by the vacuoles in the inner cytoplasmic region.

*Description of the egg after oviposition.* The laid egg of C. viridifasciata is cylindrical with rounded ends, being about 5 mm. long by 1.2 mm. thick. The ventral surface is slightly concave, the cephalic end is rounded, while the caudal end tapers slightly, as may be seen in figure 3. The egg nucleus is situated just back of the caudal end near the periphery. Grasshopper eggs of different species have been described by Carothers in 1923.

The egg is surrounded by two membranes: the chorion, a tough chitinous covering, and an inner protoplasmic membrane. The fine fibrillar and granular structure of the chorion of the laid egg may be seen in figure 28. Micropylar openings, as shown in figure 4, are numerous. They are cylindrical canals obliquely piercing the chorion and occupy a small circular area at the caudal end of the egg. (Wheeler, '89, p. 297, describes similar structures for Blatta germanica. He describes them as wide-mouthed, very oblique, funnel-shaped canals scattered over the end of the egg.) The protoplasmic membrane is a thin delicate layer about the surface of the yolk, except at the caudal end, where it is much thickened. It is also, in some eggs, thickened at the cephalic end. The reticular character of the membrane is shown in figures 23, 35, 37, 37 a, 38, 39.

Small transparent spheres containing one or more deeply staining granules are found scattered through the entire protoplasmic membrane and are usually increased in amount with the thickening of the protoplasm at the caudal and cephalic ends of the egg, as may be seen in figures 28, 32, 37 a, and 38. They are sometimes found to be of greater size in the incomplete blastoderm stage, as in figures 8 and 15, and are probably metabolic products. (Wheeler, '89, finds them numerous in the cytoplasmic membrane of Blatta eggs. In this insect they are rod-shaped masses containing deeply staining granules. They have been noted in many other insects as well.)

The yolk, centrally located, consists of semifluid masses of food material and fat-droplets. In the fixed preparations the yolk appears somewhat shrunken. Large masses are found in the cephalic and central portions, while at the caudal end the globules are small and mingle with the thickened protoplasmic area surrounding the maturation spindles. As may be seen in figure 28, they sometimes appear vacuolated.

*The maturation process.* The process of maturation consists of two mitotic divisions rapidly succeeding each other without an intervening resting stage. The chromosome number in each of the four resulting cells is one-half the original somatic number.

While the egg nucleus is generally found in the early anaphase of the first maturation division in eggs fixed immediately after the grasshopper has finished laying all the eggs in the pod, other stages up to the second maturation early anaphase were also found. The maturation divisions occur near the periphery to one side of the caudal end of the egg.

*Description of the conditions as found in C. viridifasciata.* First maturation. On the first maturation spindle twelve tetrads are to be found. Of the chromosomes in the complex, eight are large and four are small. The largest chromosome is about six times the length of the smallest. The chromosomes appear in different forms as rings, diamonds, crosses, and 8's, as in figures 24 to 27, 29 and 30. These differences result from the movements of the separating chromatids as they pass from the plane of the equatorial plate to that of the long axis of the spindle and proceed to the poles. All fiber attachments are telomitic in C. viridifasciata, as may be seen in figures 24 to 27, 29 and 30, and each tetrad is therefore made up of rod-shaped dyads. A lateral view of a first maturation early anaphase is shown in figure 24. Figures 25, 26, 29, and 30 are slightly oblique views of the same stage. Figure 27 is a polar view. They all show the full complex.

In the early anaphase the dyad character of the daughter chromosomes is clearly evident, as is seen in figure 26, in which all but three of them are completely separated. A late anaphase, shown in figures 33 and 33 a, has twelve dyads proceeding to each pole. In the first maturation telophase, figures 32, 34, and 35, the chromosomes retain their compact nature and homogeneous staining power. As no stages between this and the second maturation metaphase were observed, it remains questionable as to whether there occurs a resting condition in which all the chromosomes become diffuse.

Second maturation and polar bodies. One of the first maturation telophase groups separates off into the first polar body and remains near the periphery, the other undergoes the second maturation division. The chromosomes of the first polar body retain their dense staining capacity and compact form. They are clumped together as in figures 38 and 39, or, as in figure 37 a, are in two slightly separated groups.

In the second maturation metaphase there are twelve dyads. They are simple telomitic rod-shaped chromosomes and correspond in size seriation to the tetrads of the first maturation division. A metaphase is shown in figures 37 and 37 a. The plane of sectioning has cut the spindle in two parts. On each are six dyads. Figure 38 is another lateral view of the second maturation metaphase in which only a partial complex is shown. In figures 31 and 31 a, which are oblique views of a cut spindle on which the chromosomes are in early anaphase, the full complex is shown as it is also in figure 36.

In the telophase of the second maturation division, figure 39, there are twelve monads in the group which will form the egg nucleus. The chromosomes can be accurately counted, even though they are crowded together. The two polar bodies are seen near the periphery. The first polar body, *a*, is distinguished from the second, *b*, by its more distant position from the pronucleus and by its larger size.

No further stages were observed up to that in which twelve vesicular chromosomes are seen forming the female pronucleus, as is shown in figures 40 and 40 a and described below.

### *2. Fertilization*

In the maturation divisions the number of chromosomes in the egg nucleus has been reduced to half the number that exists in the somatic cells. A similar reduction has occurred in spermatogenesis and when the egg and sperm pronuclei unite in the process of fertilization, the resulting nucleus contains the somatic number of chromosomes.

*The sperm in the egg.* The exact time that the sperm enters the egg is unknown, although it would seem that it must be near the time of oviposition. It becomes embedded in the yolk at the caudal end not far from the oocyte nucleus, figure 3. Figure 10 represents the first stage in which the sperm was found after its entrance. It has made its way deep into the yolk of the caudal end of the egg. Here it has become cylindrically swollen, but no cytoplasm near it is as yet visible. In figure 11 a distinct cytoplasmic area has formed about the sperm, which now appears as a somewhat flattened cylinder. It is deeply staining and smooth in outline. One end tapers and there is a spiral filament at the other. In figure 7 it has become so swollen that the periphery is irregular, yet a cylindrical shape is retained with one end tapering off into a spiral, while the other end is bluntly pointed.

During these earlier stages in which the sperm is becoming vesicular, the oocyte nucleus is in the telophase of the first maturation division. The telophase represented in figure 34 was found in the same egg as the sperm shown in figure 10, and that in figure 37 was accompanied by the sperm in figure 7. Figure 12 is an extra sperm in the same egg as the one shown in figure 11. The additional sperm lies near the periphery at the caudal end of the egg. There is no cytoplasm present, and it is considerably smaller than the other functioning sperm.

The next stage observed was that of the male pronucleus, in which eleven prophase chromosomes are visible, as is shown in figures 40 and 40 a, and described below.

*The male and female pronuclei.* The stage nearest actual union of the pronuclei that was observed is shown in figures 40 and 40 a. The male and female pronuclei are embedded in a mass of cytoplasm near the caudal end of the egg. In the male pronucleus there are eleven prophase chromosomes. They are very granular and are irregular in outline. A longitudinal split is visible in some of the chromosomes. Chromatin granules are scattered throughout the nuclear network.

Within the cytoplasmic area the twelve large, discrete chromosome vesicles forming the female pronucleus are present. Chromatin granules are scattered about on a faintly staining network within each vesicle. This is the only pronuclei stage found, but the significance of the different elements is perfectly clear.

### *3. Cleavage*

*Early cleavage nuclei.* A study of the conditions in early cleavage in four- or five-day eggs of C. viridifasciata shows that a few very large nuclei are to be found scattered in the yolk. Each large, early cleavage nucleus is surrounded by an irregularly shaped cytoplasmic island of which the processes reach out among the surrounding yolk masses. The cleavage mitoses show spindles, centrosomes, and asters standing out diagrammatically. A rapid decrease in the size of the nuclei within the first few days may be noted by comparing figure 13 from a two-day egg, figures 15 and 16 from a three-day egg, and figure 14 from a four-day egg. (All drawings are of the same magnification.)

*Chromosome conditions as found in early cleavage.* Metaphase. In the metaphase the chromosomes lie in a plane perpendicular to the long axis of the spindle and midway between the poles. They are arranged radially around the spindle, the ends to which the fibers are attached are within the periphery of the spindle, while the free ends project away from the spindle in various directions. Each chromosome is separately distinct and densely staining. They graduate in size from large to small. In the male complex there is one large chromosome missing. All the chromosomes appear as rod-shaped telomitic dyads. In some of the individuals a pair of chromosomes with a pronounced constriction near the end, to which the fiber is attached, was observed and is shown in figure 15. A metaphase plate is shown in figure 13.

Anaphase. The chromosomes separate lengthwise, the halves proceeding to opposite poles. Some cases were observed in which the chromosomes, while still in the metaphase plate and in early anaphase, before the halves were completely separated, showed a longitudinal split throughout the entire length of the chromosome. Figures 15 and 16 show an anaphase. All but one of the chromosomes have completely divided and the daughter halves are going to opposite poles.

Telophase. In the early telophase the chromosomes are clumped together at the spindle poles. The ends to which the fibers are attached are close together, the free ends project in various directions. A clear area appears about each chromosome, as is shown in figure 14. This is the beginning of a vesiculation. The chromosomes expand and separate and apparently cytoplasmic fluid is imbibed. In figure 18 are shown a few chromosomes of a telophase plate in which they appear somewhat swollen and vacuolated. A polar view of a succeeding telophase stage, figure 8, shows the boundaries of each chromosome still distinct, and the chromatin forming a diffusely staining reticulum within each vesicle. A lateral view of about the same stage is shown in figure 17. In this the longitudinal split and the chromomeres are clearly distinguishable.

A late stage, and as near a resting condition as was found in the incomplete blastoderm stage, is shown in figure 20. The chromosome boundaries are still separate and the chromatin is reticular in each vesicle with deeply staining masses about in the network. These vesicles are embedded in cytoplasm and differ in this respect from another group shown in figure 19.

Prophase. The latter group (fig. 19) is perhaps a prophase stage. There are twenty-four distinct vesicles. Size seriation corresponding to that in the metaphase is indicated by the proportions of the vesicles. A large faintly staining pair, one in lateral view, *a,* and one in polar view, *b,* may be the sex pair. A peculiar funnel formation of some of the small vesicles is to be noted, and others are rounded, while the larger ones are cylindrical. Within each vesicle is a reticulum on which are scattered intensely staining chromatin granules.

Chromosome numbers. As is to be expected, the chromosomes, by their numbers, differentiate the fertilized eggs which are to produce males from those which are to produce females. There are twenty-three chromosomes in the former and twenty-four in the latter, corresponding to the spermatogonial and follicular cell counts. Figure 13 is an oblique view of a metaphase plate with twenty-four chromosomes. Also twenty-four chromosomes in a vesicular condition, in which the full complex could be accurately counted, is shown in figure 19. Figures 15 and 16, of an early anaphase (cut in two sections), show twenty-four chromosomes proceeding to each pole. An early telophase with twenty-three chromosomes is shown in figure 14.

### *4. Comparison of the metaphase chromosomes of the oocyte with those of the spermatocyte in Chortophaga viridifasciata*

Maturation of the egg and sperm are essentially the same, so far as the chromosomes are concerned, the only difference being that in the egg only one of the four groups remains in a functioning cell, while in spermatogenesis all are potentially functional. A comparison of the first and second maturation metaphase chromosomes of the oocyte with those of the spermatocyte in C. viridifasciata shows a similar compact group on the spindle, a likeness in size seriation, and a similarity of form. It is significant that the two groups of chromosomes in the maturing egg and sperm nuclei should present such a similarity, because the oocyte group is surrounded by an enormous amount of food material, while the spermatocyte, containing a small amount of cytoplasm only, is in a small cyst of about 256 like cells. It is evident that, in this case, the different environment of the two groups does not affect the chromosomal behavior.

In the first oocyte complex there are twelve tetrads, while in the first spermatocyte there are eleven tetrads and one dyad, the accessory chromosome. Thus there are two types of second spermatocytes, one containing eleven dyads, the other twelve; while all the second oocytes contain twelve dyads. In figure 41 the chromosomes are arranged in order according to size. The first two horizontal rows are slightly oblique views of the first oocyte early anaphase chromosomes and row 3 is a polar view of the same stage. It may be seen in comparing these with the lateral and slightly oblique polar views of early anaphases of the first spermatocyte chromosomes in rows 4 and 5, respectively, that the chromosomes of the two are very similar in size seriation, form, and behavior. The marked exception is the behavior and appearance of the accessory. In the first spermatocytes the unpaired sex chromosome falls among the large chromosomes. It does not divide in the first maturation division, but proceeds to one pole in advance of the other chromosomes. The periphery is irregular, and in this stage it is generally not so intensely stained as the other chromosomes. (The chromosomes at the small end of the series in row 4 appear to be larger in proportion, but they are not. This appearance is due to the fact that they are drawn in lateral view and the full length of the chromosome is visible. Those in row 5, from another cell of the same cyst, are in polar view, while those in rows 1, 2, and 3 are in oblique view.)

In the second maturation division the oocyte chromosomes, as shown in the oblique views in rows 6 and 7, there is a similarity in all respects to the spermatocyte chromosomes shown in rows 8 and 9. Row 8 is a spermatocyte complex with twelve chromosomes and row 9, one with eleven chromosomes. There is no differential behavior of the accessory chromosomes in the second spermatocytes or in the second oocytes.

Thus it may be seen from this study that the chromosomes contributed by the two parents in fertilization are comparable and that the cells in preparation for this union undergo a like series of changes in so far as the chromosomes are concerned.

### 5. *A comparison of the metaphase chromosomes of the oocyte in Circotettix verruculatus with those in the spermatocyte in C. verruculatus and with those in the oocyte in Chortophaga viridifasciata*

By way of comparison with the conditions in C. viridifasciata, the chromosomes in the first maturation division in the eggs of C. verruculatus may be considered. Although the chromosomes in the male germ cells of the two species have been known to be dissimilar, the fact that a dissimilarity also exists in the females is significant.

In C. verruculatus, the first spermatocyte complex, as described by Carothers in 1921 (p. 462), consists of four large atelomitic chromosomes, the atelomitic accessory, two medium and one small telomitic chromosomes, and three identifiable pairs, the members of which may vary from individual to individual, having terminal fibers in some and subterminal or median in others. In all individuals, one of the large atelomitic chromosomes is an octad multiple, involving what would otherwise be a larger and a smaller telomitic tetrad. This reduces the number of separate elements to eleven in the spermatocytes and the somatic number in the male to twenty-one instead of twenty-three chromosomes. In the female complex there are two accessories, as is apparent from a study of the somatic cells.

In the eggs of C. verruculatus that were sectioned for the present study, three first maturation metaphases and a first maturation early telophase as is shown in figures 21, 22, 23, and 28, respectively, were observed. There are eleven chromosomes in figure 21. Figures 22 and 23 do not show the full complex. Seven of the chromosomes are large, two of them medium, and two small. In this genus some of the chromosomes differ in form from those of Chortophaga, due to the fact that some of them are atelomitic. Four large atelomitic rings with median fiber attachments are to be seen in figure 23, while there are three in figure 21. Two other atelomitic chromosomes with subterminal fiber attachments are present in figure 21, and the chromosome in the upper right position is heteromorphic. A constriction of the chromosomes is seen in the large cross-shaped chromosome of figure 23. In the early telophase, figure 28, the chromosomes retain their compact nature as in C. viridifasciata.

The similarity that exists between the chromosomes of the oocyte with those of the spermatocyte in C. viridifasciata, a condition also existing in C. verruculatus, is added proof of the constancy in the chromosome complex of each species.

## REVIEWS AND DISCUSSIONS

### *Oogenesis in insects*

The maturation stages in insects have received little attention compared to that given spermatogenesis, due to the difficulty in handling eggs heavily laden with yolk. Polar-body formation has been observed in many insects, although a detailed cytological study of the process is generally lacking. A complete review of the literature on maturation in insect eggs was given by Morrill in 1909. Since that time a few papers have appeared, but most of these consider the chromosomes only as condensed elements. (Many papers dealing with oogenesis among parthenogenetic forms have appeared, but as they have no direct bearing on the present paper they are not reviewed. The reader is referred to

Wilson's "The cell in development and heredity," 1925, for a thorough review of the subject.)

### *Review of papers dealing with orthopteran oogenesis*

Four papers which describe meiosis in the orthopteran egg have appeared. Blochman ('87) and Wheeler ('89) report the conditions as found in Blatta germanica; von Baehr ('07) gives them for Bacillus rossii, and Pehani ('25) for Carausius morosus.

Wheeler ('89) and Blochman ('87), in describing polar-body formation in Blatta germanica, give similar accounts, and the following review considers them together. In the young transparent ova when the nucleus has reached its acme of development in volume, it is a proportionately large spherical body, more highly refractive than the surrounding cytoplasm. In its achromatic network are suspended small irregular particles of chromatin, or they may be aggregated in one or two large masses. The nuclear wall is very distinct. In the following stages the nucleus becomes amoeboid and moves to the surface of the egg on the concave side. The chromatin loses its staining capacity, gives off 'maturation spheres' (Wheeler's observation), and decreases in size. The first polar spindle is at right angles to the surface of the egg. The chromosomes divide and the first polar body is constricted off, the second polar body being similarly produced. The polar bodies later disintegrate. The female pronucleus increases in size and leaves the surface of the egg. It exhibits a coiled chromatin filament, while the male pronucleus is granular. No astral radiations are seen. The two pronuclei fuse in the middle of the yolk about one-third of the dorsoventral diameter from the back of the egg. The cleavage nucleus divides, forming amoeboid cells, some of which migrate to the surface to form the blastoderm. The chromosome numbers were not determined and the observations are of little cytological value with regard to them. The figures of both Blochman and Wheeler are diagrammatic and show no cytological details.

Polar-body formation in Bacillus rossii, a parthenogenetic form, was described by von Baehr in 1907. The egg nucleus lies in the peripheral protoplasm in the posterior half on the ventral side of the egg, and at oviposition is preparing to undergo the first maturation division. In the metaphase eighteen to twenty chromosomes are counted. They are in the form of tetrads, a few appear as dyads, or tetrads with a weak linkage of the dyads. The first polar body is not separated from the egg, but remains in the protoplasmic membrane. In interkinesis the chromosomes in each nucleus arrange themselves in an equatorial plate, the egg nucleus to form the second polar body and the first polar body to divide. These chromosomes appear clearly as dyads. After division, which does not take place simultaneously in the two groups, the chromosomes appear as monads. The egg nucleus now stains lightly and increases quickly to many times its original size. It lies not far from the periphery and here the first cleavage takes place. The figures show no cytological details and the work is of little value with regard to the chromosomes.

Pehani ('25) describes oogenesis in Carausius morosus. The nucleus of an egg fixed immediately after laying may be found in one of the various stages from the first maturation even to the metaphase of the second maturation. The earliest stage found in the laid egg was a spireme prophase of the first maturation division with the chromosomes interlaced. When in the equatorial plate the diploid number appears, but the chromosomes are difficult to count. There are approximately sixty-two to sixty-four short, almost spherical rods. The position of the spindle on the surface of the egg is variable. It may be parallel or oblique, but is seldom perpendicular. No centrosome is present. Only a small amount of cytoplasm surrounds the spindle, and it is soon filled with yolk spheres.

In interkinesis the egg nucleus shows no resting structure. The chromosomes, easily differentiated, remain scattered in the nuclear cavity. They are still difficult to count, but it is evident that more than the haploid number is present. Several chromosomes group themselves together in this stage, and when they arrange themselves in an equatorial plate for the second maturation division, some remain banded together in chain form or are grouped around a central chromosome. The diploid number in the second maturation is certain. The nucleus enlarges, sinks in the egg, and the first cleavage follows. The method of blastoderm formation was not observed. Examination of Pehani's figures that show polar and lateral views of early anaphases of the first and second maturation divisions would indicate a poor fixation of the material, which therefore is not sufficiently accurate to afford a knowledge of the cytological details. The material is also unfavorable for the study of chromosomes.

### *Discussion of orthopteran oogenesis*

It may be seen from the above reviews that the incompleteness of observations prevents a thorough comparison to the conditions as found in the grasshopper. Perhaps the most significant fact to be noted from them is that no diffuse state of the chromatin in the resting stage between the maturation divisions is described in any of the animals, and this also appears to be lacking in the grasshopper. A dissimilarity in the structure of the female pronucleus in Blatta germanica, Bacillus rossii, and in Carausius morosus from that in the grasshopper exists. In the former a typical resting nucleus is found, while in the latter it is one in which the vesicular chromosomes remain separately embedded in the cytoplasm. Also, it is to be noted that the plane in which the polar spindles are formed varies, being perpendicular, oblique, or parallel to the surface of the egg in Carausius morosus and in the grasshopper, but it is always perpendicular in Blatta germanica.

In general, it may be said that in oogenesis in the Orthoptera studied there is a growth period of the ovarian oocytes in which the egg nucleus becomes greatly enlarged and vesicular. At the time of oviposition the maturation divisions are initiated, there being two rapidly succeeding divisions in which the chromosome number is reduced to half. The chromosomes are in the form of tetrads resulting in the haploid number in the first maturation metaphase, except in Carausius morosus, in which the diploid number of the chromosomes in dyad form is found. The diploid number is also present in the second maturation metaphase in this case. In the others the haploid number of chromosomes, in dyad composition, is found in the second maturation metaphase. The two polar bodies separate off into the peripheral protoplasm and are non-functional. The egg pronucleus enlarges and sinks into the egg. In Carausius morosus and Bacillus rossii, which are parthenogenetic forms, it immediately undergoes the first cleavage; but in Chortiphaga viridifasciata and Blatta germanica it unites with the male pronucleus, and the resulting zygote then undergoes the first cleavage.

### *Recent papers on oogenesis in other orders of insects*

Of recent studies among other groups of insects which are of cytological value, that of S. H. Schrader's in 1924 on a parasitic Strepsipteran, Achroschismus wheeleri Pierce, is the only one that considers the chromosomes in other stages than those in which they are condensed elements. Briefly reviewed, the resting oocyte nucleus has a very fine reticulum studded with chromatin granules. At the beginning of maturation eight condensations of chromatin appear and are surrounded by a clear zone. The clear non-staining material draws out to form a sheath about each tetrad. The nuclear membrane shrinks and the fusiform bodies come together at one end. Fan-shaped figures are formed, and finally a compact barrel-shaped figure results. There is no trace of fibrillar formation to the sheath. At the entrance of the sperm the sheath disappears, the chromosomes divide, and there appears to be a fibrillar character to the sheath. The two polar bodies are formed in rapid succession. After the second polar body is extruded, the chromosomes become vesicular and stain lightly, the chromatin granules adhere to the membrane of each chromosome.

The sperm enters at any point, but once entered it is oriented toward the female pronucleus. One or more sperm may enter, but only one functions. The sperm reaches the nucleus at the time of the extrusion of the second polar body. The tail disappears, the head swells and becomes a typical resting nucleus. A single fusion nucleus is formed and in the first somatic division sixteen chromosomes appear.

This paper is of especial interest in that it describes the only other case observed in which the female pronucleus resembles that in the grasshopper. A stage is figured in which the female pronucleus, consisting of separated chromosome vesicles, is embedded in the same mass of cytoplasm as the male pronucleus. This is a stage comparable to that described and figured for the grasshopper in the present paper. In these two cases direct evidence of the genetic continuity of the chromosomes from the first maturation division of the oocyte to the pronucleus stage is obtained. There is no resting stage, in which the chromosomes apparently lose their identity, between the first and second maturation divisions or after the second maturation division before the union of the pronuclei.

Two papers dealing with oogenesis in Lepidoptera may be mentioned. They consider the chromosomes mainly as condensed elements and stress points with relation to their numbers and behavior. Seiler ('12, '14) studied three species. In Phragmatobia fuliginosa twenty-eight chromosomes are found in the first maturation metaphase. There are twenty-seven short rod-shaped chromosomes and one chromosome which is considerably larger. In the first maturation anaphase groups the separated mates of the large chromosome are found to be unequal in size. In the group in which the smaller chromosome appears, an extra small chromosome has been split off, accounting for the decrease in size of the mate. This results in a dimorphism in the eggs. Half have twenty-eight chromosomes, the rest have twenty-nine. All sperm contain twenty-eight chromosomes. At fertilization embryos contain fifty-six or fifty-seven chromosomes, the former producing males and the latter females. In Lymantria japonica and Lymantria dispar thirty-one chromosomes, both in the egg and sperm, are present, and there are sixty-two somatic chromosomes in all embryos. Thus for these two species Seiler assumes an x-y sex-determining pair.

Dederer ('15) observed the two maturation divisions in Philosamia cynthia. The twenty-six somatic chromosomes are rounded bodies of approximately equal size. In the reduced number they divide equally and simultaneously in the first and second maturation divisions.

In a study on certain coreid Hemiptera, Morrill ('09) observed maturation in two species, Anasa tristis and Protenor belfragei. In these the eggs all contain the same number of chromosomes. Sexual differences are due to the presence of an accessory chromosome, and in these cases the sperm are dimorphic. Thus embryos of fourteen and fifteen chromosomes are found in Protenor, and of twenty-one and twenty-two in Anasa. In two other species (in which maturation was not observed) embryos of two types having twenty-one and twenty-two chromosomes in Chelinidae vittigera and fifteen and sixteen in Archimerus alternatus were also found.

In Diptera, Huettner ('24) describes maturation in Drosophila melanogaster. The ovarian egg has a large reticular nucleus, but when ready to be fertilized the metaphase spindle of the first polar body is present. Polar-body formation is initiated by the entrance of the sperm. The first polar body is pushed off in a direction opposite to the male pronucleus. No resting nucleus appears, but the two diploid groups form new spindles. The second polar body is produced and the first polar body divides. Four haploid chromosome groups are now found in the telophase, the three polar bodies and the female pronucleus. The male and female pronuclei fuse, a resting nucleus being formed. The polar bodies finally disintegrate. No chromosome count is given and the figures show little detail with regard to them. However, the paper is chiefly concerned with the origin of the germ cells.

Oogenesis in the different insects, no matter how much the details of the process may vary, accomplishes one thing, i.e., the reduction of the chromosome number from the diploid to the haploid count. The widespread occurrence of sex-determining chromosomes among insects as found from studies on male germ cells is further demonstrated by examination of the meiotic divisions in the female germ cells, although the studies in the latter field are comparatively meager.

### *Significance of chromosome vesicles*

The vesicular condition of the chromosomes is indicative of their individuality. In all diffuse stages of the chromosomes in early cleavage in the grasshopper egg the chromosome boundaries could be recognized. It is of particular interest that a perceptible chromosome individuality is maintained in these cells, because some of them are destined to become primordial germ cells. In many insects the latter can be identified with certainty in early cleavage and traced onward to the formation of the gonads. A determination of the origin of the germ cells in the grasshopper is being undertaken at the present time by a student in this laboratory. It is possible that this study will establish a visible continuity of individual chromosomes from the meiosis of the oocyte to the differentiation in early cleavage of those cells which are to become the primordial germ cells. This would indicate real independence of the germ line.

The occurrence of chromosome vesicles is common in the eggs of animals. Richards ('17) has traced the chromosomes in the eggs of Fundulus from the metaphase of one-cell generation through all the stages of mitosis and interkinesis as continuous structures which give rise to the prophase chromosomes of the next mitosis. However, he does not give a count of the chromosomes establishing a numerical constancy as is done in the present paper for the grasshopper. Richards gives a list showing the wide distribution of the condition and a general summary of the occurrence of chromosome vesicles.

Chromosomes in a vesicular condition have also been found in the Orthopteran testis. Sutton ('00) describes vesicles in Brachystola magna. In the primary spermatogonial divisions, immediately after the formation of the partition wall, the chromosomes begin to disintegrate. They lose their homogeneous appearance and become granular. At the same time there is found about each one a distinct membrane. Separate chromosome vesicles, however, do not last long, as their walls are soon dissolved where they come in contact at their polar ends. The chromatin of the vesicular chromosome, after remaining some time at the periphery, again recedes and accumulates at the center of the vesicle. The vesicular character of the accessory is also noted, and it remains independent throughout its entire existence.

Pinney ('08) describes vesicles in Phrynotettix magnus which were thought to remain distinct through the resting stage. As the chromosomes move apart each becomes surrounded by a delicate membrane. The chromosome vesicles then enlarge and the chromatin becomes diffuse.

Wenrich ('16), working on the same material, uses as proof of chromosome individuality the presence of vesicles which retain their own limits in the resting nucleus. This is associated with a constancy of the finer organization of the chromosomes, both from stage to stage and from one individual to another. Davis ('08) describes vesicles in Steiroxys trilineata that fuse at one end and the accessory is enclosed in a separate vesicle.

Thus it may be seen that orthopteran material, especially of the family Acrididae, offers considerable proof of chromosome individuality through the presence of chromosome vesicles.

### *Chromosomes in orthopteran spermatogenesis*

A detailed study of the chromosomes in orthopteran spermatogenesis was published by McClung in 1914. Each individual animal shows in every cell the same chromosome complex. The number, size, form, and behavior, due to an exact division of each chromosome in every mitosis, are held constant for each species. The relative sizes of the chromosomes in the complex of each species vary and range from small to large, either gradually or by abrupt steps.

Chromosome form is determined by degree of condensation, mode of fiber attachment on the spindle, by multiplicity, and by other conditions. In the Acrididae, some tetrads may be rod-shaped, others crosses, diamonds, rings, double rings, or V's, double V's, J's, L's, or I's in shape. As may be expected, the metaphase chromosomes of the haploid and diploid series of a species vary in form. Thus the rod-shaped dyads in the spermatogonia, when united in pairs in the first spermatocyte metaphase, may form rings, crosses, diamonds, etc., as in the cells of Chortophaga figured in the present paper.

The rod-shaped chromosome, as is characteristically displayed by Mecostethus, has a terminal fiber attachment. This is the most common type of orthopteran chromosome. In division a rod is split lengthwise, the halves going to opposite poles. When the fiber attachment is atelomitic, other forms are produced. In the large rings of Circotettix (Carothers, '21) the fiber attachment is median, while in the J-shaped chromosome it is subterminal on one dyad, terminal on the other.

Further differences in form and an apparent reduction in number of the chromosomes are revealed by a study of the multiples of Hesperotettix and Mermiria by McClung in 1917. In Mermiria a hexad multiple, involving the accessory and a large tetrad, produces a J-shaped chromosome. In Hesperotettix octad multiples involving two tetrads are formed, producing rings or V's. In both cases the telomitic fiber attachments remain unchanged. With the formation of one, two, or three multiples in Hesperotettix, the number of separate elements in the first spermatocyte may be eleven, ten, or nine, respectively, instead of twelve chromosomes. However, it is significant that each chromosome of a multiple retains its identity and organization in every detail and the reduction in number is only apparent. In comparing chromosome complexes, therefore, it is always necessary to consider their history.

From this brief review of the character of the chromosomes in spermatogenesis compared to their history in oogenesis, it is seen that they display constancy in the male and female complexes and are strictly comparable. In regard to the accessory, it may be stated that it is found paired in the female germ cells studied and falls among the group of large chromosomes.

## SUMMARY

1. A technique is developed for the preparation of grasshopper eggs for cytological study. In brief, the eggs are fixed in Carnoy-Lebrun and infiltrated with celloidin, in which serial sections are made.
2. Oogenesis from the late ovarian stages to the completion of meiosis is studied in Chortophaga viridifasciata.
3. The egg nucleus while in the ovary passes through a growth period in which it increases greatly in size and the nuclear structures become very diffuse.
4. Meiosis takes place after oviposition.
5. In the first maturation early anaphases twelve telomitic tetrads are found. All chromosomes have terminal fiber attachments in C. viridifasciata.
6. Of the two telophase chromosome groups of the first maturation, one separates off in the first polar body, the other undergoes a second maturation division.
7. In the second maturation metaphases and early anaphases twelve dyads are found.
8. Of the two telophase chromosome groups of the second maturation division, one separates off in the second polar body, the other forms the egg pronucleus.
9. The sperm enters the egg near the time of oviposition. It becomes embedded in the yolk and forms the male pronucleus.
10. The male and female pronuclei are later seen embedded in a single mass of cytoplasm near the caudal end of the egg.

11. In early cleavage, the chromosomes, by their numbers, differentiate male- and female-producing eggs. There are twenty-three chromosomes in the former and twenty-four in the latter.

12. In early cleavage the chromosomes assume a vesicular condition in the telophase, retaining their individual boundaries in all stages of interkinesis.

13. A comparison of the first and second maturation metaphase chromosomes of the oocyte with those of the spermatocyte in Chortophaga viridifasciata shows a similarity in most respects. In the female complex of the first maturation there are twelve tetrads, while in the male complex there are eleven tetrads and one dyad. The latter, or accessory chromosome, is unusual in its behavior.

14. A comparison of the first maturation metaphase chromosomes of Chortophaga viridifasciata with those of Circotettix verruculatus shows a dissimilarity of conditions with regard to the chromosome forms and numbers. In the latter some of the chromosomes are atelomitic and an octad multiple is formed.

15. The dissimilarity of the chromosome conditions in the eggs of these two species also obtains for the male germ cells.

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# EXPLANATION OF PLATES

The drawings were made with the aid of a camera lucida, using a 20 × compensating ocular and a Spencer homogeneous immersion 1.8-mm. objective, N.A. 30. The photomicrographs were made with a 2-mm. Zeiss objective, N.A. 40, and a 4 projection ocular.

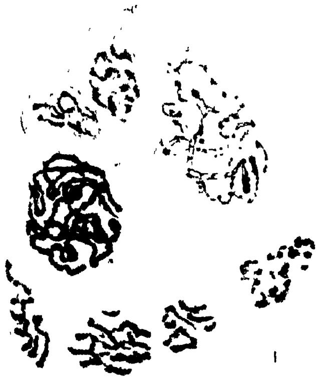
All drawings except those noted below were originally magnified 3040 times and are reduced to 1520 in reproduction. Figures 1, 2, and 5 are reduced to 1013. Figure 41 is reduced to 2533. Figure 3 was magnified 20 times and reduced to 7 times; figure 4, 528 times and reduced to 176; figure 6, 840 times and reduced to 280. The photomicrographs were made at a magnification of 1200 and reduced to 750.

Figures 21, 22, 23, 28, 46, 47, and 49 are from Circotettix and all others from Chortophaga.

## PLATE 1

### EXPLANATION OF FIGURES

1 Distal end of an ovarian tubule showing two young oocytes.
2 An oocyte nucleus in an egg ready for oviposition.
3 Diagram of an egg after oviposition.
4 Micropylar structures in the caudal end of an egg.
5 An oocyte nucleus in the growth period.
6 Follicular cells.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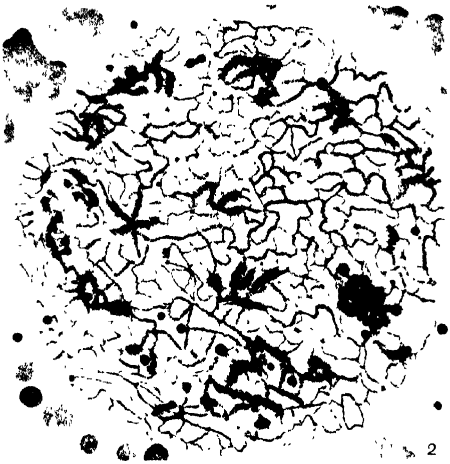
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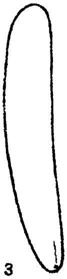
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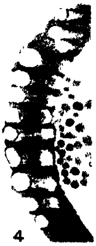
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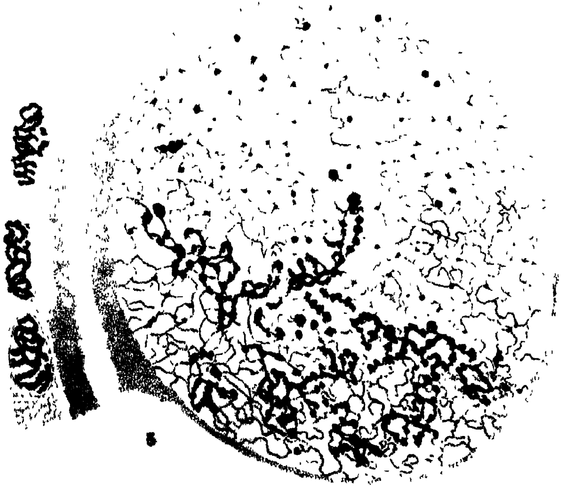
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## PLATE 2

### EXPLANATION OF FIGURES

7 A functional sperm in an egg (fig. 32)

8 Polar view of a telophase of an early cleavage cell.

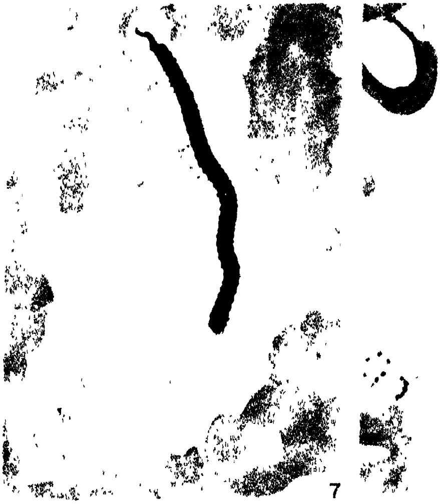
9 Polar view of a metaphase of a follicular cell, showing the full somatic complex of twenty-four chromosomes.

10 A functional sperm embedded in the yolk (fig. 34).

11 A functional sperm in an egg (fig 12).

12 A non functional sperm in an egg.

13 An oblique view of a metaphase from a two day egg, showing twenty-four chromosomes.



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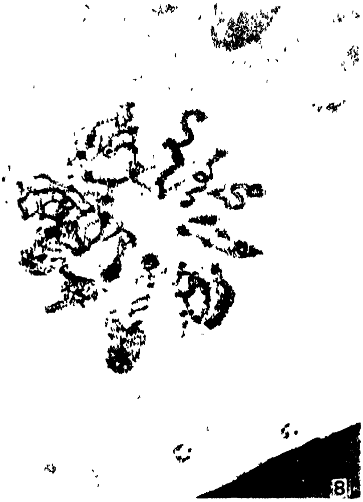

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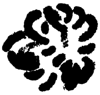

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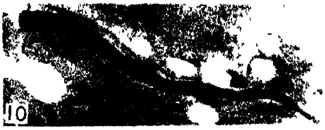

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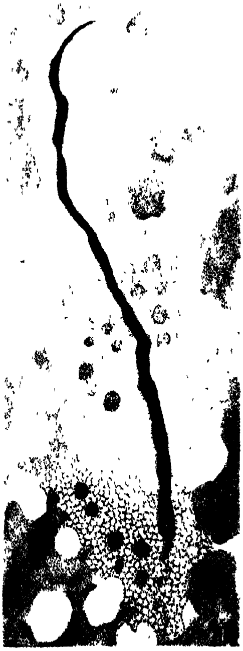

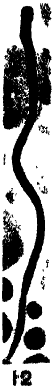

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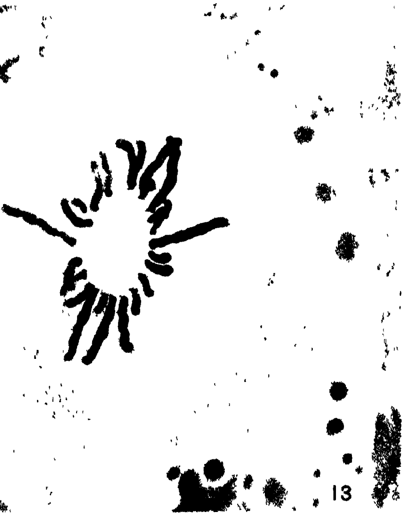

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## PLATE 3

### EXPLANATION OF FIGURES

14 A telophase from a four day egg showing twenty three chromosomes

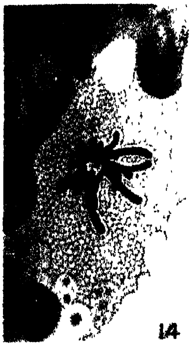
15 and 16 An oblique view of an anaphase, showing twenty four chromosomes proceeding to each pole. (Cut in two sections.)

17 A lateral view of a telophase of an early cleavage cell. A longitudinal split of some of the chromosomes can be distinguished.

18 A telophase of an early cleavage cell, showing only a few of the chromosomes.

19 A prophase of vesicular chromosomes in early cleavage There are twenty four vesicles, *a* and *b* are more lightly staining than the others and are perhaps the sex pair.

20 As near a resting stage as was found in eggs of the incomplete blastoderm stage.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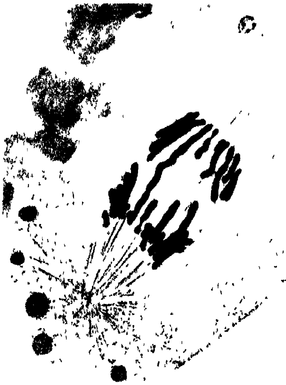
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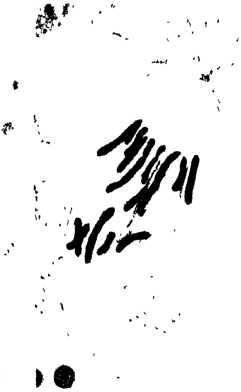
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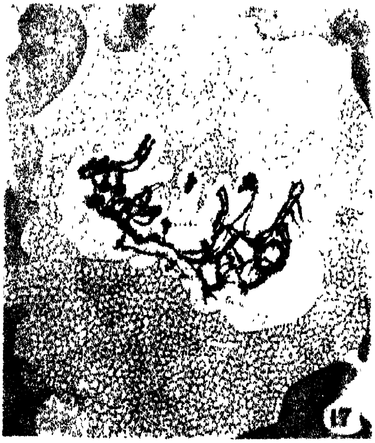
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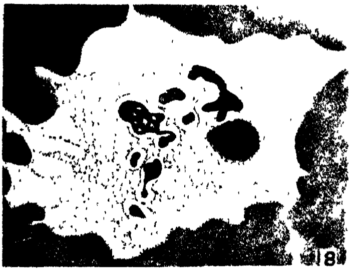
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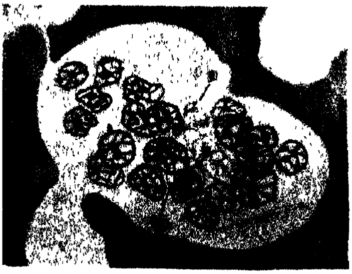

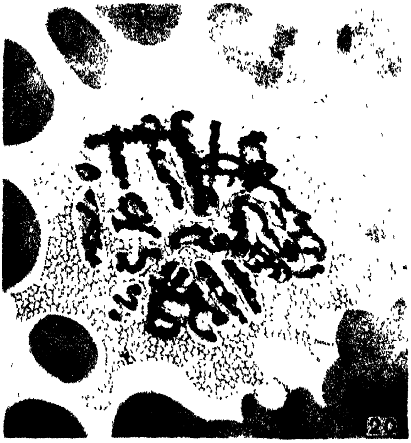
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## PLATE 4

### EXPLANATION OF FIGURES

21 An obliquely lateral view of a metaphase of the first maturation division, showing the full complex.

22 The same A partial complex.

23 The same. A partial complex.

24 A lateral view of an early anaphase of the first maturation division, showing the full complex.

25 The same in an obliquely lateral view.

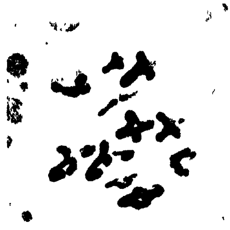
26 The same as figure 25 All but three of the dyads are completely separated.

27 The same stage in polar view, showing the, full complex.

28 An early telophase of the first maturation division

29 An obliquely lateral view of an early anaphase of the first maturation division, showing the full complex.

30 The same.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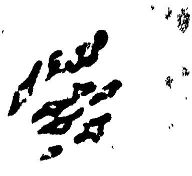
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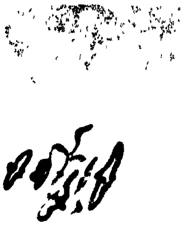
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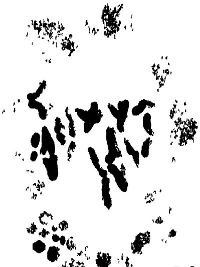
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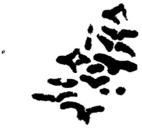
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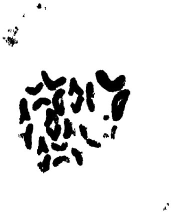
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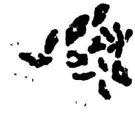
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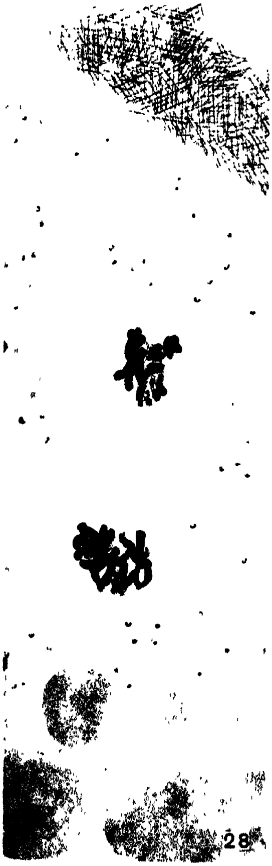
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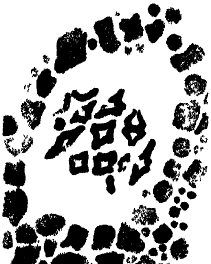
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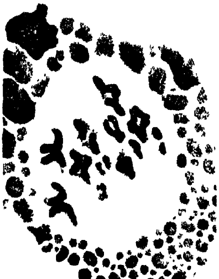
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# PLATE 5

## EXPLANATION OF FIGURES

31 and 31a An oblique view of an early anaphase of the second maturation division, showing the full complex (Cut in two sections.)

32 A telophase of the first maturation division. (In the same egg with the sperm shown in fig. 7.)

33 and 33a An oblique view of a late anaphase of the first maturation division, showing twelve dyads proceeding to each pole. (Cut in two sections.)

34 A telophase of the first maturation division. (In the same egg with the sperm shown in fig. 2.)

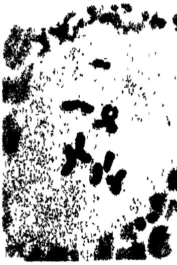
35 A first maturation telophase.

36 An oblique view of an early anaphase of the second maturation, showing the full complex.

37 and 37a A lateral view of the metaphase of the second maturation, showing the first polar body. The full complex is shown. (Cut in two sections.)

38 An oblique view of the metaphase of the second maturation, showing the first polar body. (Cut in two sections and only one shown.)

39 The egg pronucleus and the two polar bodies. The first polar body is larger and in a more distant position from the egg pronucleus than the second polar body.



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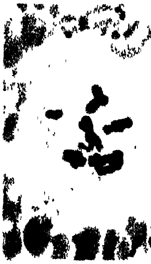

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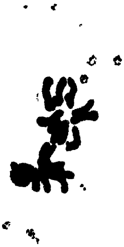

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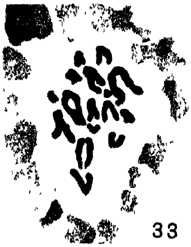

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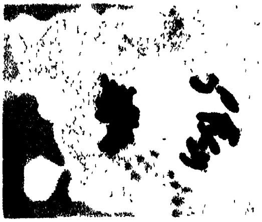

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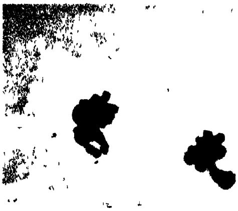

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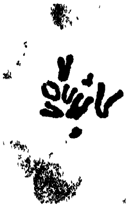

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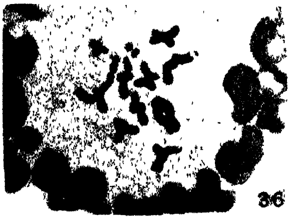

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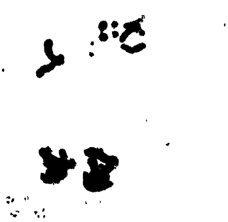

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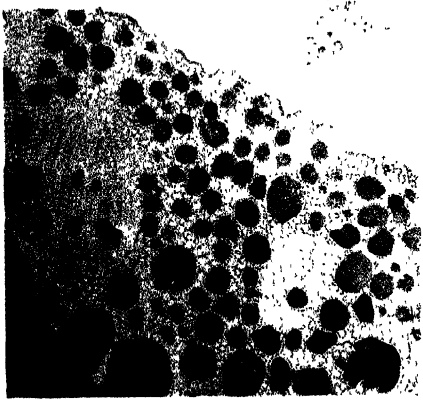

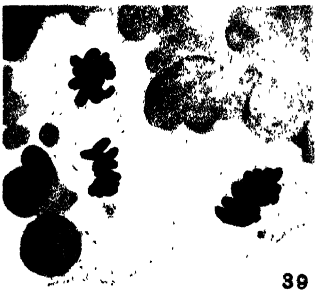

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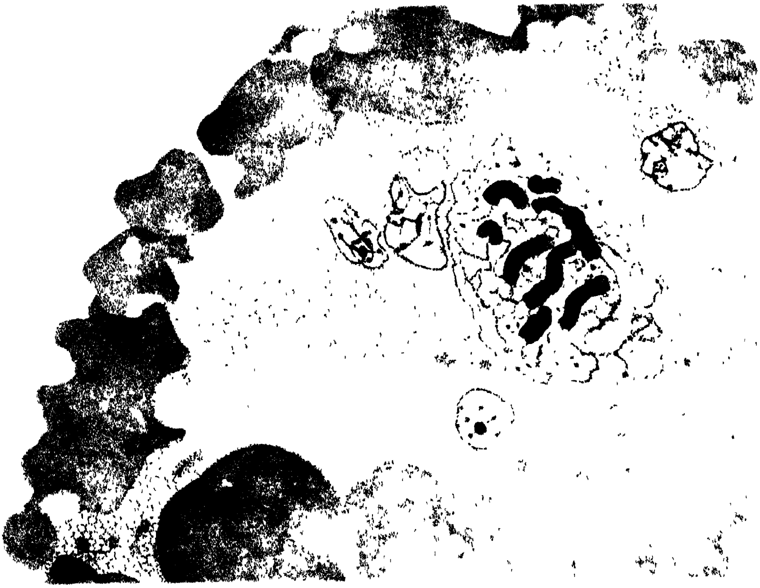
## PLATE 6

### EXPLANATION OF FIGURES

40 and 40a The male and female pronuclei. (Cut in two sections.)



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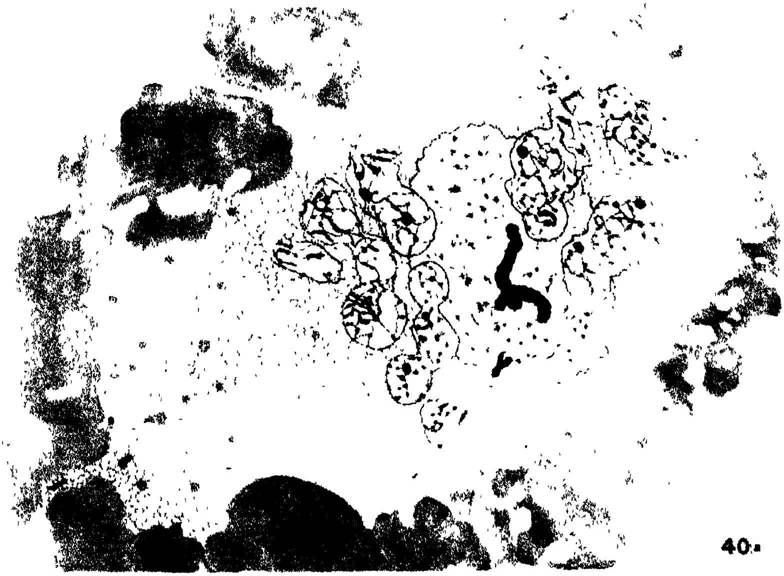


40a

## PLATE 7

### EXPLANATION OF FIGURES

41 Early anaphase chromosomes of the first and second maturations:

Rows 1 and 2 Oblique views of first oocyte complexes.

Row 3 The same in polar view.

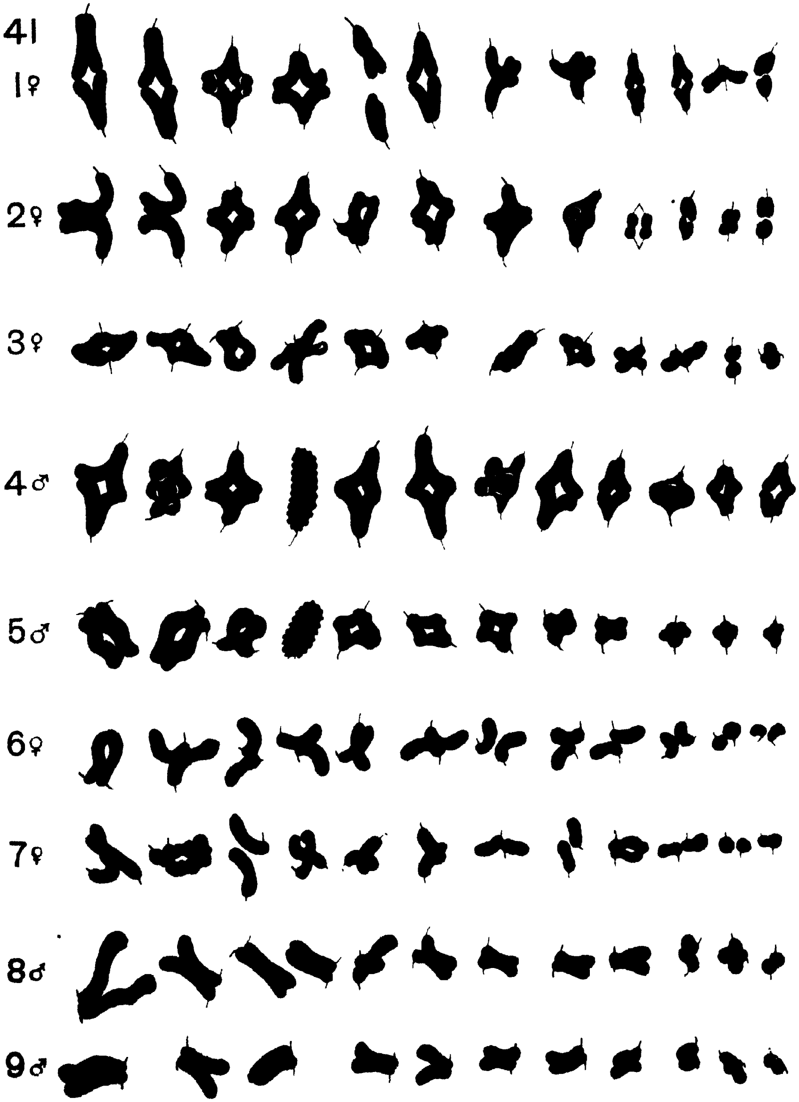
Row 4 Lateral view of the first spermatocyte complex.

Row 5 The same in an obliquely polar view.

Rows 6 and 7 Oblique views of second oocyte complexes.

Row 8 Oblique view of a second spermatocyte complex with twelve chromosomes.

Row 9 The same with eleven chromosomes.


41
1♀
2♀
3♀
4♂
5♂
6♀
7♀
8♂
9♂

## PLATE 8

### EXPLANATION OF PHOTOMICROGRAPHS

42 The male and female pronuclei. Portions of two vesicular chromosomes and the large male pronucleus are shown (figs. 40 and 40 a).

43 A lateral view of a first maturation metaphase.

44 The same at a different focus.

45 The same as figure 42 at a different focus.

46 A first maturation early telophase, showing one of the daughter groups of chromosomes (fig. 28).

47 The same showing the other chromosome group.

48 A first maturation early anaphase, showing a large tetrad (fig. 24).

49 A first maturation metaphase, showing three large tetrad rings (fig. 23).

50 A first maturation telophase (fig. 32).

51 A telophase of the second maturation.

52 A late anaphase of the first maturation.

53 The same at a different focus.

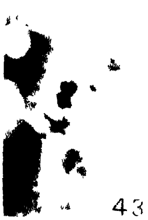
54 The same as figure 42 at a different focus. Portions of six of the vesicular chromosomes of the female pronucleus are visible.

55 A lateral metaphase of the second maturation, showing the dyad character of the chromosomes.

56 The same as figure 55 at a different focus. The first polar body is visible in this section.

57 A functional sperm in an egg (fig. 5).

42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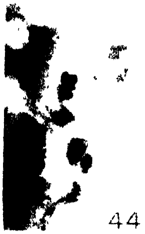
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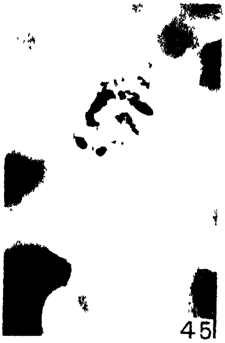
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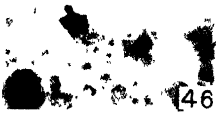
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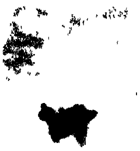
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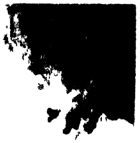
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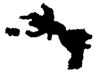
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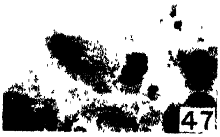
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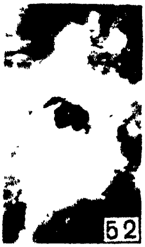
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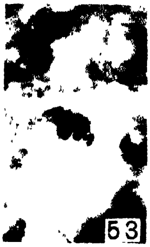
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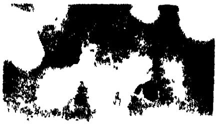
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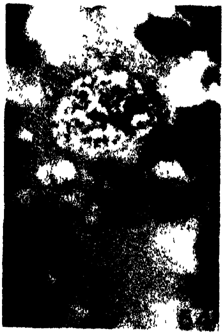

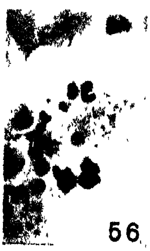
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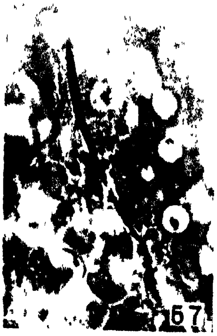
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## PLATE 9

### EXPLANATION OF PHOTOMICROGRAPHS

58 Lateral view of an early cleavage metaphase.

59 The same in an oblique view in a younger egg (fig. 11).

60 An early telophase of an early cleavage cell, showing the vesicular condition of the chromosomes.

61 The same in polar view at a little later stage (fig. 13).

62 A lateral view of an early telophase of an early cleavage cell, showing the longitudinal split in one of the chromosomes in focus (fig. 17).

63 Another telophase group in which the chromosome split is visible.

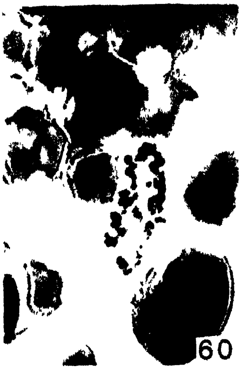
64 A resting stage in which the chromosomes retain their separate boundaries (fig. 20).

65 Vesicular prophase chromosomes in an egg in the early cleavage stage (fig. 19).

66 The same as figure 65 at a different focus.

58

59



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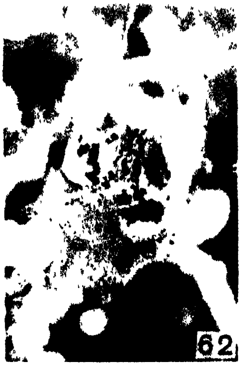

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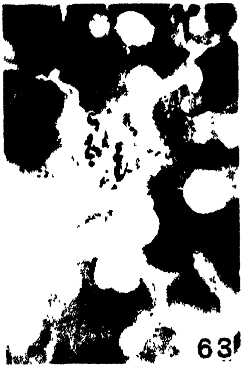

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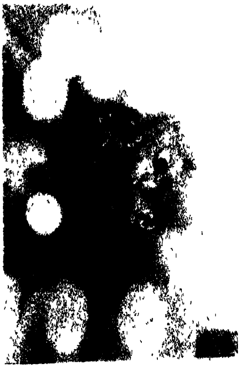

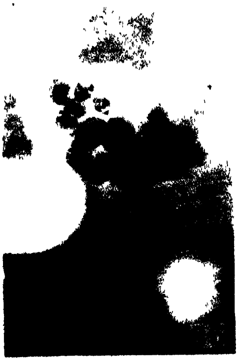

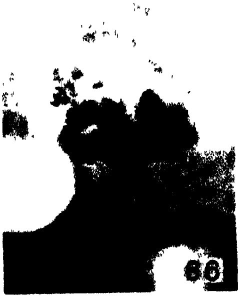

66

# THE EFFECTS OF CERTAIN PHYSICAL AND CHEMICAL FACTORS ON LOCOMOTION AND OTHERS LIFE-PROCESSES IN AMOEBA PROTEUS[1]

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*The Zoological Laboratory of the Johns Hopkins University*

FOUR FIGURES

AUTHOR'S ABSTRACT

Amoeba proteus was raised in a modified and diluted Ringer solution. When the pH of this culture medium became less than 6.0, the normal activities of the amoebae were interfered with, and when a still lower pH was attained, the amoebae died off. The same was true when the pH became greater than 8.0. At neutrality the activities were subnormal, very dark, and rounded. The rate of locomotion of amoebae raised in solutions with a pH less than 7.0 showed a maximum rate of locomotion at pH 6.6, which decreased as the pH changed in either direction, dropping to a very low rate at pH 7.0 and above and also below 6.0. For amoebae raised at a pH above 7.0 the rate was maximum at pH 7.6 and decreased as the pH changed in either direction, it was low at pH 7.0 and below and also above 8.0.

On increasing the external osmotic pressure of the medium it was found that the effects caused varied somewhat with the hydrogen-ion concentration. Small increases in osmotic pressure decreased the rate from the normal at pH 6.0 and 8.0, increased it at pH 6.6 and 7.6 and did not affect it at pH 7.0. Osmotic pressures above that produced by M/20 lactose caused locomotion to cease in a short time at all pH values.

## I. HYDROGEN-ION CONCENTRATION

### INTRODUCTION

Strong concentrations of either acids or bases are fatal to protozoa. Collett ('19) maintains that different acids kill various protozoa at a concentration of 0.0008 N. or less. The results of Woodruff and Bunsel ('09) and of Dale ('13) on Paramoecium agree closely with those of Collett and, in addition, show that alkalies are toxic in approximately the same concentrations as acids. Crane ('21) finds that paramoecia can live twenty-four hours in any hydrogen-ion concentration between pH 5.0 and 7.6. Saunders ('24) gives a very limited range of hydrogen-ion concentrations within which Spirostomum thrives and multiplies, namely, pH 6.0 to 7.6, with an optimum at pH 7.3. The same author ('25) maintains that paramoecia adhere to each other, to glass, and to débris more readily in alkaline than in acid solutions, the optimum for adherence being pH 7.9. Beers ('25) concludes that there is no specific relation between the hydrogen-ion concentration and encystment in Didinium nasutum, and Kater and Burroughs ('26) come to the same conclusion in reference to Polytomella citri.

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[1] This investigation was suggested by Prof. S. O. Mast and carried out under his direction. He has also furnished valuable aid in the presentation of the results. For these favors, I am greatly indebted to him.

Edwards ('23) found that with amoebae immersed in M/500 KCl local application of weak acid or alkali on the anterior surface causes the formation of a protuberance at the point of application. Pantin ('23) found that certain marine amoebae thrive best in sea-water of pH 8.2 or thereabout and ('26) that the addition of certain acids to sea-water until a pH of 7.0 to 6.0 is attained, or the addition of alkalies until a pH of 10.0 is reached, causes movement to cease. Taylor ('24) asserts that Amoeba proteus in acid cultures is very clear and in alkaline cultures very dark, that it thrives best at a hydrogen-ion concentration of pH 6.6, and that it cannot be raised at a pH below 3.0 or above 7.0.

It therefore appears that there is a hydrogen-ion concentration close to neutrality which is optimum for the activities of most protozoan forms, and that extreme deviations from this concentration in either the alkaline or the acid direction are fatal. There appears to be much diversity as to the exact location of the optimum, it being pH 7.3 for Spirostimum (Saunders), pH 6.6 for Amoeba proteus (Taylor), and pH 8.2 for marine amoebae (Pantin). In the following pages we shall present results obtained in a detailed investigation of the relation of the hydrogen-ion concentration of the surrounding medium to the activities of a fresh-water amoeba, Amoeba proteus.

## MATERIAL AND METHODS

### *Material*

Amoeba proteus[2] was employed in this work. The stock was derived from collections made from a small stream near the Zoölogical Laboratory of the Johns Hopkins University.

All chemicals used were specially purified by several recrystallizations by Hynson, Westcott & Dunning, druggists, of Baltimore. The water used in making the solutions was distilled in an ordinary block-tin still; then redistilled after adding $H_2SO_4$ and $KMnO_4$,; and again after adding $Ba(OH)_2$. The redistilling apparatus was made entirely of pyrex glass. The water was allowed to drop from the condenser into a pyrex flask exposed to air fairly free from dust.

For the determination of the hydrogen-ion concentration a La Motte apparatus 5B was used. This is a colorimetric apparatus which was devised and described by Brown ('24) and perfected by La Motte & Co., of Baltimore. The special features of this apparatus are: 1) the color comparisons are made in small glass cells of equal volume and shape, on a white background; 2) the same solution of dye is used in the standard buffers as in the solutions to be tested; 3) less than 1 cc. of fluid is required to make an individual test, and, 4) an individual test can be made in less than two minutes.

### *Culture methods*

Two distinct methods of culturing amoebae were used. The first. culture method A, may be described as follows: A solution designated modified Ringer solution I was prepared as indicated in table 1. One liter of this solution was then poured into a pyrex-glass flask, which was placed into a vessel containing water and heated until the water in the vessel boiled, then 2 grams of short pieces of timothy hay were added to the solution in the flask, allowed to remain ten minutes, after which the source of heat was removed, the hay taken from the flask and discarded. When the solution had cooled, it was poured in equal amounts into ten 100-cc. pyrex-glass flasks, inoculated with organisms (bacteria and Chilamonas chiefly) taken from a flourishing amoeba culture that had passed through a no. 50 filter-paper, corked tightly with cotton, and allowed to stand for a week or more. During this time the fluid first becomes acid, then alkaline. The changes in acidity and alkalinity observed in five different cultures of this kind are given in table 2. These changes are typical for all fresh cultures made by this method. When the hydrogen-ion concentration desired was attained by cultures in

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[2] The term Amoeba proteus is used in the older sense.

TABLE 1

*Modified Ringer solution I. A synthetic solution for culturing Amoeba proteus*

| | | |
|---|---|---|
| NaCl | .1166 gram | .002000 mol. |
| KCl | .00466 gram | .000063 mol. |
| $CaCl_2$ | .00400 gram | .000036 mol. |
| $KH_2PO_4$ | .06084 gram | .000507 mol. |
| $NaHCO_3$ | .00660 gram | .000790 mol. |
| Water to 1000 cc. | | |
| Total | | .003396 mol. |

TABLE 2

*Showing changes in hydrogen-ion potential in culture fluid A, during the first nine days after being made up. Note that the cultures turn distinctly acid on the second and third days and that after that they gradually become alkaline*

| SUCCESSIVE DAYS | CULTURES | | | | |
|---|---|---|---|---|---|
| | a | b | c | d | e |
| 1 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 |
| 2 | 6.5 | 6.3 | 6.5 | 6.4 | 6.3 |
| 3 | 6.9 | 6.7 | 6.7 | 6.8 | 6.7 |
| 4 | 7.1 | 7.0 | 7.1 | 7.1 | 7.0 |
| 5 | 7.2 | 7.2 | 7.2 | 7.2 | 7.2 |
| 6 | 7.4 | 7.4 | 7.3 | 7.3 | 7.3 |
| 7 | 7.4 | 7.4 | 7.4 | 7.4 | 7.4 |
| 8 | 7.6 | 7.6 | 7.6 | 7.6 | 7.5 |
| 9 | 7.8 | 7.6 | 7.6 | 7.6 | 7.6 |

their return to alkalinity, they were inoculated with amoebae. Then each day fresh culture fluid, made up in the same fashion as the original, was added in such quantity that the tendency of the fresh fluid to become acid neutralized the tendency of the cultures to become alkaline.[3]

It was found that in this way a fairly constant hydrogen-ion concentration could be maintained. If a concentration below pH 7.0 is desired, it can be obtained by adding a constant amount of fresh fluid each day,[4] but if a concentration above pH 7.0 is desired, it was found that the amount of fresh fluid added each day had to be varied considerably. In table 3 are recorded the results of four attempts to maintain a constant hydrogen-ion concentration, two at pH 7.5, cultures E and R, and two at pH 6.8, cultures B17 and C20. Owing to the fact that the food supply was not sufficient and too variable, this method was not successful in raising amoebae when the hydrogen-ion concentration was maintained above 7.0. Culture method A was, therefore, used only to culture amoebae on the acid side of neutrality. Amoebae raised by this method will be referred to in the following pages as 'acid amoebae.'

The second method of culturing amoebae, culture method B, was extremely simple, and may be described as follows: Modified Ringer solution I was poured into a finger-bowl and a few stems of timothy hay, about 1 cm. long, added. This fluid first slowly becomes slightly acid and then alkaline. When pH 7.5 to 7.7 is attained, the alkaline and the acid tendencies generally come to an equilibrium automatically. When this hydrogen-ion concentration was attained, the cultures were inoculated with amoebae. Amoebae flourish well at pH 7.5 to 7.7 in these cultures. This method was, therefore, used for culturing amoebae on the alkaline side of neutrality. Amoebae raised in this culture fluid will be called 'alkaline amoebae.'

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[3] The volume was maintained constant by pouring out at the same time old culture fluid.

[4] Slight variation in the area of the surface exposed so as to vary the rate at which $CO_2$ escaped was occasionally necessary.

## RELATION BETWEEN THE HYDROGEN-ION CONCENTRATION AND THE CULTURE OF AMOEBA PROTEUS

We shall now describe two experiments which bring out clearly the relation between the hydrogen-ion concentration and the culture of adult Amoeba proteus in modified Ringer solution I. The results obtained in these experiments are typical examples of what was repeatedly observed.

In the first experiment an acid culture was taken in which the hydrogen-ion concentration had been maintained at pH

TABLE 3

*Showing changes in hydrogen-ion potential for twenty-two successive days in 100 cc. of culture fluid A, to which a certain amount of fresh culture fluid A was added daily*

| SUCCESSIVE DAYS | CULTURES | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | E | | R | | B 17 | | C 20 | |
| | pH | Amount culture added in cc. | pH | Amount culture added in cc. | pH | Amount culture added in cc. | pH | Amount culture added in cc. |
| 1 | 7.8 | | 8.0 | 8.0 | 6.9 | 10 | 6.9 | 10 |
| 2 | 7.8 | 7.5 | 7.9 | 15.0 | 6.7 | 10 | 6.7 | 10 |
| 3 | 7.5 | 0.0 | 7.5 | 10.0 | 6.7 | 10 | 6.9 | 10 |
| 4 | 7.7 | 3.5 | 7.4 | 5.0 | 6.9 | 10 | 6.9 | 10 |
| 5 | 7.7 | 7.0 | 7.5 | 5.0 | 6.8 | 10 | 6.7 | 10 |
| 6 | 7.7 | 15.0 | 7.5 | 5.0 | 6.8 | 10 | 6.8 | 10 |
| 7 | 7.4 | 5.0 | 7.5 | 5.0 | 6.8 | 10 | 6.8 | 10 |
| 8 | 7.4 | 5.0 | 7.5 | 5.0 | 7.0 | 10 | 6.8 | 10 |
| 9 | 7.5 | 3.0 | 7.5 | 5.0 | 6.8 | 10 | 6.8 | 10 |
| 10 | 7.5 | 4.0 | 7.5 | 5.0 | 6.8 | 10 | 6.8 | 10 |
| 11 | 7.4 | 2.0 | 7.6 | 8.0 | 6.7 | 10 | 6.7 | 10 |
| 12 | 7.6 | 5.0 | 7.2 | 0.0 | 6.7 | 10 | 6.8 | 10 |
| 13 | 7.5 | 3.0 | 7.5 | 3.0 | 6.8 | 10 | 6.7 | 10 |
| 14 | 7.5 | 3.0 | 7.5 | 4.0 | 6.8 | 10 | 6.7 | 10 |
| 15 | 7.5 | 3.0 | 7.5 | 3.0 | 6.8 | 10 | 6.8 | 10 |
| 16 | 7.5 | 3.0 | 7.5 | 5.0 | 6.8 | 10 | 6.8 | 10 |
| 17 | 7.5 | 3.0 | 7.5 | 4.0 | 6.8 | 10 | 6.8 | 10 |
| 18 | 7.4 | 3.0 | 7.5 | 3.0 | 6.8 | 10 | 6.6 | 10 |
| 19 | 7.4 | 2.0 | 7.5 | 3.0 | 6.7 | 10 | 6.7 | 10 |
| 20 | 7.5 | 3.0 | | | 6.8 | 10 | 6.8 | 10 |
| 21 | 7.4 | 2.0 | | | 6.7 | 10 | 6.7 | 10 |
| 22 | 7.5 | 3.0 | | | 6.8 | 10 | 6.8 | 10 |

6.6 to 6.8 (definitely acid) for two weeks or more. The amoebae were in adult condition, multiplying rapidly by fission. The surface of the culture was now increased and a few short stems of hay were added to it. In a short time the alkalinity began to increase slowly, and as it increased, the amoebae gradually became dark and more sluggish in their movements. When the pH reached 7.2, all had become very dark and nearly spherical and they seemed to have decreased considerably in number. As the increase in alkalinity continued, the condition of the amoebae began to improve. When pH 7.4 was reached, nearly all were again normal in appearance, moving about and feeding actively; but when pH 7.8 was reached, they had again become dark and sluggish, though not spherical. Later, when pH 8.5 had been obtained, the amoebae had disappeared.

In the second experiment an alkaline culture was selected in which the pH had been maintained at 7.5 to 7.7 for two weeks or more. The amoebae were in adult condition and they were multiplying rapidly by fission. In this experiment fresh culture fluid was added each day in such amounts that a gradual decrease in alkalinity was obtained. The changes in the amoebae were essentially the same as those observed in the first experiment. As the alkalinity decreased to pH 7.2, the amoebae became more and more opaque and more and more sluggish in their movements. When it reached pH 7.0, most of them had become very dark and nearly spherical. As the decrease continued to pH 6.8, they again became active and began to multiply. The pH was held now at 6.8 for three weeks. During this time the amoebae were active and they became large and abundant. Finally, the acidity was slowly increased from pH 6.8 to 6.0. During this time the amoebae gradually decreased in number until all disappeared after the hydrogen-ion concentration reached pH 6.0.

These results seem to indicate that the modified Ringer solution used is optimum for growth and reproduction in Amoeba proteus when the pH is 6.6 to 6.8 or 7.5 to 7.7; that, if the solution is at or near neutrality, it causes striking changes in the physiological conditions which apparently are detrimental to the life-processes, and that strong acid and alkaline solutions are unfavorable.

### RELATION BETWEEN HYDROGEN-ION CONCENTRATION AND RATE OF LOCOMOTION IN AMOEBA PROTEUS

To ascertain the effect of any given factor on the rate of locomotion in Amoeba, it plainly is necessary to vary this factor in a known way without varying any other factors. This cannot be accurately done in culture fluids, for the compositions of such fluids are not accurately known. For this reason, all of the observations on the rate of locomotion were made in a balanced salt solution in which amoebae live and move readily. This solution will be designated 'modified Ringer solution II.' Its composition is given in table 4. It differs from culture fluids A and B as follows: 1) Sodium bicarbonate is eliminated. 2) The phosphate buffers are more concentrated, and the hydrogen-ion concentration is adjusted as desired by adding KOH to $KH_2PO_4$. 3) The $CaCl_2$ content is one and one-half times as high. 4) It contains no hay abstract. 5) The molecular and the osmotic concentrations are, respectively, 0.001210 mole and 0.001283 mole greater, not taking into consideration the hay abstract. The differences referred to under (5) are, however, at least partially eliminated by the substances extracted from the hay.

TABLE 4

*Modified Ringer solution II. A synthetic solution used to ascertain the relation between the rate of locomotion and hydrogen-ion concentration in Amoeba proteus*

| | | |
|---|---|---|
| NaCl | .11660 gram | .002000 mol. |
| KCl | .00466 gram | .000063 mol. |
| $CaCl_2$ | .00600 gram | .000054 mol. |
| $KH_2PO_4$–KOH buffer 50 cc. of M/20 solution | | .00250 mol. |
| Water to 1000 cc. | | |
| Hydrogen-ion concentration depends upon the ratio of $KH_2PO_4$–KOH added. | | |
| Total | | .004617 mol. |

Modified Ringer solution II was made up in the following manner: 1) A stock solution containing $CaCl_2$, NaCl, KCl, each thirty times as concentrated as indicated in table 4, was made up. 2) A series of phosphate buffers of different hydrogen-ion concentrations were made up according to Clark ('22), with the exception that KOH was used instead of NaOH[5]. 3) Then 3.33 cc. of the first solution and 5.00 cc. of a buffer solution having the desired hydrogen-ion concentration were put into a 100-cc. volumetric flask and water added to make 100 cc. In this way modified Ringer solution II can be made with any hydrogen-ion concentration desired within the range covered by these buffers, and with the acidity and alkalinity as the only essential variables.

The effect of varying the pH on the activities of Amoeba was ascertained by the use of modified Ringer solution II as follows:

An individual test consisted in removing a well-fed amoeba from a culture, transferring it through three changes of modified Ringer solution II with the desired hydrogen-ion potential, allowing it to remain fifteen minutes in the last for adjustment, and then obtaining the rate of monopodal locomotion by projecting, with a camera lucida, the image of the individual under observation onto a record sheet and at minute intervals making sketches of the posterior end. The distance thus obtained was 131 times the actual distance the amoeba traveled.

All experiments were carried out in a dark room at temperatures between 21° and 24°C.

The effect of the hydrogen-ion concentration on acid amoebae raised in culture fluid A and alkaline amoebae raised in culture fluid B was investigated as follows: Amoebae were taken from each of two cultures: one which had been at a hydrogen-ion concentration of pH 6.6 to 6.8 for two weeks or more, and another which had been at one of pH 7.5 to 7.7 the same length of time. The acid culture was treated in such a way as to produce an increase in the hydrogen-ion concentration to pH 7.0, and the alkaline culture in such a way as to produce an increase to 7.2. Both were held at these concentrations for two days, then the amoebae, one at a time, were transferred to each of a number of dishes, each containing modified Ringer solution II, which differed in hydrogen-ion potential from that in the others. Observations were now made on the form and rate of locomotion in these solutions as previously described. In this way the average rate of locomotion for five acid amoebae for fifteen minutes each was ascertained in modified Ringer solution II with each of the following hydrogen-ion concentrations: 6.0, 6.6, 7.0, 7.2, and 7.6; and for five alkaline amoebae each with each of the following hydrogen-ion concentrations: 8.0, 7.6, 7.2, 7.0, and 6.6. Observations were not made later than two

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[5] Clark's procedure is to add to 50 cc. of M/5 $KH_2PO_4$, M/5 NaOH, until the desired pH is obtained and then to dilute to 200 cc. with distilled water. The resulting molecular concentration of the buffer phosphates is M/20.

TABLE 5

*Relation between the rate of locomotion in acid and alkaline amoebae and hydrogen-ion concentration. Temperatures, 21 to 24°C. A, acid amoebae; B, alkaline amoebae*

| SPECIMEN | HYDROGEN-ION CONCENTRATION | | | | | |
|---|---|---|---|---|---|---|
| | 6.0 | 6.6 | 7.0 | 7.2 | 7.6 | 8.0 |
| | Rate of locomotion | | | | | |
| A 1 | 1.50 | 2.02 | .81 | .96 | .97 | |
| A 2 | 1.57 | 1.60 | .81 | .83 | .78 | |
| A 3 | 1.51 | 2.04 | .94 | .92 | .89 | |
| A 4 | 1.42 | 1.63 | .82 | 1.13 | | |
| A 5 | 1.76 | 1.36 | .91 | .97 | | |
| Average | 1.56 | 1.73 | .86 | .94 | .88 | |
| B 1 | | .77 | 1.10 | | 1.53 | 1.72 |
| B 2 | | | .64 | .97 | 1.54 | 1.70 |
| B 3 | | | .60 | .96 | 1.75 | 1.54 |
| B 4 | | | .68 | 1.04 | 1.88 | 1.99 |
| B 5 | | | .86 | .90 | 1.66 | 1.75 |
| Average | | .77 | .77 | .97 | 1.67 | 1.75 |

hours after the amoebae were placed into a given solution, and each amoeba was used in only one solution.

The results obtained are presented in table 5 and figures 1 and 2. By referring to table 5 and figure 1, it will be seen that for amoebae from the acid culture, beginning at a hydrogen-ion concentration of pH 6.0, the rate of locomotion increased to a maximum at 6.6, then fell abruptly to a minimum at 7.0, after which it remained nearly constant; while for amoebae from the alkaline culture, beginning at a hydrogen-ion concentration of pH 8.0, the rate was at a maximum, then dropped to a minimum at 7.2, after which it remained nearly constant. It is therefore evident that the effect of the hydrogen-ion concentration depends upon the constitution of the culture in which the amoebae have been grown. If the culture is alkaline, the maximum rate of locomotion is on the alkaline side of neutrality, and if it is acid, the maximum rate is on the acid side; neutrality appears to be unfavorable, no matter whether the amoebae were grown in acid or alkaline cultures.

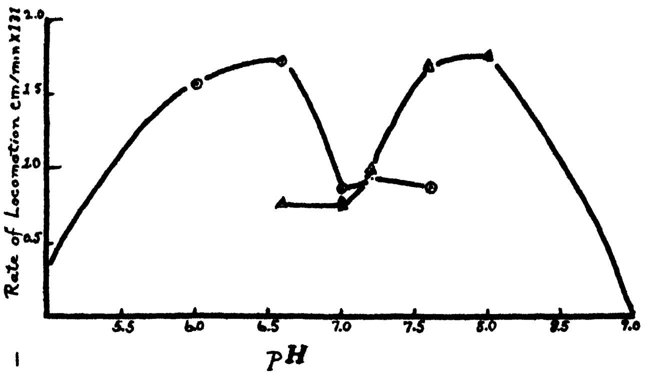

Fig. 1 Curves, showing the relation between rate of locomotion in Amoeba proteus and hydrogen-ion potential. ⊙, amoebae from acid cultures; △, amoebae from alkaline cultures. Rate of locomotion in terms of centimeters per minute times 131 plotted as ordinates, hydrogen-ion potential plotted as abscissas. The portions of the curves below, pH 6.0, and above, pH 8.0, are hypothetical.

The diagrams in figure 2, A, represent degree of opacity, general form, and appearance typical of amoebae in the different solutions. The forms assumed by acid amoebae at pH 6.0, were almost exclusively monopodal and cylindrical. The same statement holds for alkaline amoebae at pH 8.0.

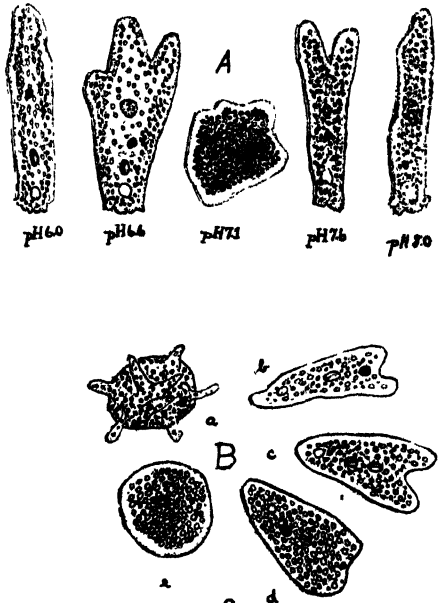

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Fig. 2 Diagrams showing the form, the degree of opacity, and the type of locomotion of amoebae in modified Ringer solution II. A, acid amoebae in pH 7.1, 6.6, and 6.0, and alkaline amoebae in pH 7.1, 7.6, and 8.0. B, successive forms assumed by acid and alkaline amoebae at pH 7.1. *a*, immediately after transfer to the solution; *b*, ten to fifteen minutes later; *e*, final form.

In pH 6.6 acid amoebae are very fluid and the protoplasm flows in broad streams. The same is true of alkaline amoebae in pH 7.6. At pH 7.0 both acid and alkaline amoebae were nearly spherical and apparently fluid; the supporting structure evidently had almost, if not entirely, broken down.

If either acid or alkaline amoebae are placed in modified Ringer solution II of pH 7.1 and left a sufficient length of time, they tend to become spherical and lose almost entirely all evidences of life. Shortly after being placed in this solution, they assume almost a spherical form, with the exception that numerous, short, slender pseudopods protrude in all directions. On remaining in the solution, they resume locomotion again, but, as time passes, they become lobose and darker in color, and then nearly spherical and very dark (fig. 2, B).

The time that amoebae must remain in modified Ringer solution II of pH 7.1 before a spherical form is assumed varies greatly. In the preceding experiments the time required in amoebae from both cultures, acid and alkaline, was two or three hours, but it will be remembered that the cultures from which these amoebae came were brought to a pH of 7.0 to 7.2 two days before the experiments were performed. If amoebae are taken from an acid culture, pH 6.8, and placed directly in this solution, it requires five or six hours, but amoebae from alkaline cultures, pH 7.6, require from ten to twenty-four hours. The length of time required seems to depend upon the composition of the culture media from which the amoebae come.

The question now arises as to whether the amoebae that have almost lost their power of locomotion in a solution of pH 7.1 regain it if they are put into a solution having a higher or lower hydrogen-ion concentration.

If amoebae are taken from an alkaline culture, pH 7.6, and placed in a solution of pH 7.1, left until locomotion has all but ceased and then placed into a solution of either pH 8.0 or 6.0, there is an immediate resumption of locomotion. If amoebae from an acid culture, pH 6.8, are treated in like manner, then placed in a solution of pH 6.0, there is a marked increase in the rate of locomotion; but, if they are placed in a solution of pH 8.0, no perceptible increase occurs.

Examples may be cited: 1) Two amoebae from an alkaline culture, pH 7.6, were placed in modified Ringer solution II, pH 7.1, the rate of locomotion in each was less than 0.5. One of these amoebae was then placed in a modified Ringer solution II, pH 8.0; in ten minutes the rate was 1.55. The other amoeba was placed in a modified Ringer solution II, pH 6.0; in a few minutes it attained an average rate of 2.40. 2) Two amoebae from an acid culture, pH 6.8, were placed in modified Ringer solution II, pH 7.1, and left until the rate of locomotion decreased to 0.5 or less. One of these was then transferred to modified Ringer solution II, pH 6.0; the rate increased gradually to 2.86. The other amoeba was transferred to modified Ringer solution II, pH 8.0; no increase in rate of locomotion took place.

In table 6 the results of a number of similar experiments are brought together. Acid and alkaline amoebae were placed in modified Ringer solution II, pH 7.1, left there until locomotion had decreased to 0.5 or less; then they were transferred to modified Ringer solution II, pH 6.0, 6.6, 7.6, or 8.0, as indicated in the table. Each value in the table is the average rate per minute obtained for five minutes of monopodal locomotion of one amoeba.

These results indicate very clearly that the activities of amoebae in modified Ringer solution II, pH 7.1, are definitely depressed, and that this depressing effect, if it is not very great, is removed, partially at least, by increasing either the hydroxyl or the hydrogen-ion concentration.

Can the depressing effect be removed by the same means if it continues until locomotion has ceased entirely?

Attempts were made with both acid and alkaline amoebae to answer this question, and it was found that if the amoebae which have come to rest in modified Ringer solution II, pH 7.1, are transferred to one of pH 8.0, locomotion is not resumed, but that if they are transferred to one of pH 6.0, it is resumed, provided that they are transferred not later than two days after locomotion has ceased. If transferred later than this it is not resumed in any solution.

The effect of locally increasing or decreasing the hydrogen-ion potential was studied in the following manner: A small drop of modified Ringer solution II, pH 7.1, containing an amoeba which had ceased to move altogether, was placed on the bottom of a dry and chemically clean Syracuse watch-glass. Near this drop was placed a drop of modified Ringer solution II, pH 6.0 or 8.0. Then with a clean needle a narrow projection of fluid was drawn out from each drop toward the other until they nearly touched; then the amoeba in the drop was brought up against the surface layer at the end of the projection, after which the two projections were united. As soon as the modified Ringer solution II, pH 6.0 or 8.0, came

TABLE 6

*Relation between rate of locomotion and hydrogen-ion concentration in acid and alkaline amoebae which had been subjected to modified Ringer solution II, pH 7.1 until locomotion had dropped to a rate below 0.5, and then transferred to solutions with higher or lower pH. Temperature 21 to 24°C.*

| SPECIMEN | HYDROGEN-ION CONCENTRATION | | | |
|---|---|---|---|---|
| | 6.0 | 6.6 | 7.6 | 8.0 |
| Alkaline amoebae | Rate of locomotion | | | |
| 1 | 2.30 | 1.15 | 2.10 | 1.80 |
| 2 | 3.03 | 1.16 | 1.70 | 1.70 |
| 3 | 3.52 | 1.80 | 2.40 | 1.70 |
| 4 | | 1.80 | 2.80 | 1.50 |
| 5 | | | | 1.40 |
| 6 | | | | 1.48 |
| 7 | | | | 1.59 |
| 8 | | | | 1.36 |
| 9 | | | | 1.16 |
| 10 | | | | 1.36 |
| Average | 2.59 | 1.48 | 2.25 | 1.50 |
| Acid amoebae | | | | |
| 1 | 2.50 | | | 0 |
| 2 | 2.12 | | | 0 |
| 3 | 2.04 | | | 0 |
| 4 | 1.32 | | | |
| 5 | 1.32 | | | |
| Average | 1.86 | | | 0 |

in contact with the amoeba, a pseudopod was thrown out in the direction of contact. This reaction is remarkable in that it is caused both by an increase in either hydrogen- or hydroxyl-ion concentration, the solutions in the drops being otherwise identical. Edwards ('23), as stated previously, obtained the same for local increase in acids or alkalies on the surface of amoeba.

## DISCUSSION

Our results in culturing amoebae on the acid side of neutrality agree essentially with those of Taylor ('24), who found that Amoeba proteus used in her investigations thrived best at a pH of 6.6, that a rise in pH to 7.0 or above caused a depression in the rate of growth and in the activities of the amoebae, and that she was unable to culture them successfully at pH 7.0 or above. The results presented above demonstrate, however, that Amoeba proteus can be successfully cultured at pH 7.6 as well as at pH 6.6. She probably had conditions similar to ours; that is, rather concentrated salt solutions compared with those in which Amoeba proteus generally lives. Consequently, when her amoebae were forced to go through neutral conditions the depressing effect which we have observed was experienced, resulting in a dark, rounded, and sluggish condition. Her failure to obtain growth on the alkaline side of neutrality was probably due to insufficient time for adjustment.

The results presented above indicate that the rate of locomotion of Amoeba proteus depends upon the hydrogen-ion concentration in the same way as the metabolic activities.

The question now arises: is there any explanation for the fact that both acid and alkaline amoebae do not thrive or locomote well at neutrality? It may be associated with the peculiar properties of water at neutrality. Again, it may be that the iso-electric condition of some important protein in amoeba occurs at this point, resulting in the inactivation of this protein and thus causing the depression observed. Or it may be that in salt solutions, such as the ones used above, attachment of the amoebae to the substratum is hindered at neutrality, owing to an unfavorable electrical condition existing between the amoebae and the glass or between the amoebae and the water.

## II. OSMOTIC PRESSURE

### INTRODUCTION

Very little work has been done on the problem of the relation between external osmotic pressure and the rate of locomotion of Amoeba. Mast ('23, '26) appears to hold that the cell contents in Amoeba are hypertonic resulting in turgidity, and that, when there is no movement, the elastic strength of the plasmagel and the plasmalemma plus the external osmotic pressure equal the internal osmotic pressure. He also holds that the outward pressure exerted by the hypertonicity of the cell contents gives rise to the formation of a pseudopod if the elastic strength of the plasmagel is locally reduced and implies that locomotion is related to the water content. If this is true there should be a specific relation between rate of locomotion and external osmotic pressure.

Pantin ('23) observed in a species of marine amoebae that movement is impossible in concentrated and in diluted sea-water, and he concludes that high and low external osmotic pressures inhibit locomotion. He did not, however, make detailed measurements covering the relation between the concentration of the sea-water and the rate of locomotion, and he did not exclude the possibility of effects other than osmotic pressure in the dilute and the concentrated sea-water used.

The specific relation existing between locomotion in Amoeba and osmotic pressure, then, has been defined only in an indefinite and unsatisfactory way. It was to clear up this important problem that the experiments described in the following pages were undertaken.

### MATERIAL AND METHOD

The relation between external osmotic pressure and the rate of locomotion in Amoeba proteus was ascertained as follows:

Lactose[6] was added to three portions of modified Ringer solution II of a given hydrogen-ion potential until the molecular concentrations of lactose in the three portions were: M/40, M/20, and M/10, respectively. About twenty-five amoebae were taken from a culture, passed through three changes of modified Ringer solution II with the desired hydrogen-ion concentration (without lactose) and allowed to remain for fifteen minutes in the last, after which the rate of locomotion was ascertained as described above. Then about one-fourth of these amoebae was put into each of three Syracuse watch-glasses full of Ringer solution II containing lactose, M/10, M/20, and M/40, respectively, and the remaining one-fourth was left as a control in the solution without lactose.

The dish containing M/10 lactose was now selected and the rate of locomotion of amoeba in this solution recorded for five minutes. This was then repeated for an amoeba in the M/20 and M/40 lactose solutions, respectively, after which it was successively repeated for each of the three solutions for two hours. At the end of this time, the four watch-glasses were covered and placed in a moist chamber. The amoebae were then transferred every few hours to fresh solutions to prevent error due to excessive fermentation and evaporation. During the following two days at various intervals observations were made on the rate of locomotion of the amoebae in the four watch-glasses.

This experiment was repeated with acid amoebae in each of the three concentrations of lactose in modified Ringer solution II at pH 6.0, 6.6, and 7.1; and with alkaline amoebae in the same concentrations of lactose at pH 7.1, 7.6, and 8.0. The results obtained are presented in figures 3 and 4.

By referring to figure 3, the following will be seen:

The rate of locomotion of acid amoebae in the modified Ringer solution at pH 6.0 and of alkaline amoebae at pH 8.0 varies inversely with the concentration, clearly indicating that it is specifically related to and varies directly with the water content of the amoebae.

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[6] Lactose was used because it is non-toxic, a non-electrolyte, a substance of large molecular weight, and a sugar which is fermented only by Bacterium coli.

In the solutions at pH 6.6 and 7.6 the rate of locomotion of acid and alkaline amoebae evidently does not vary inversely with the concentration, there being, during the first three

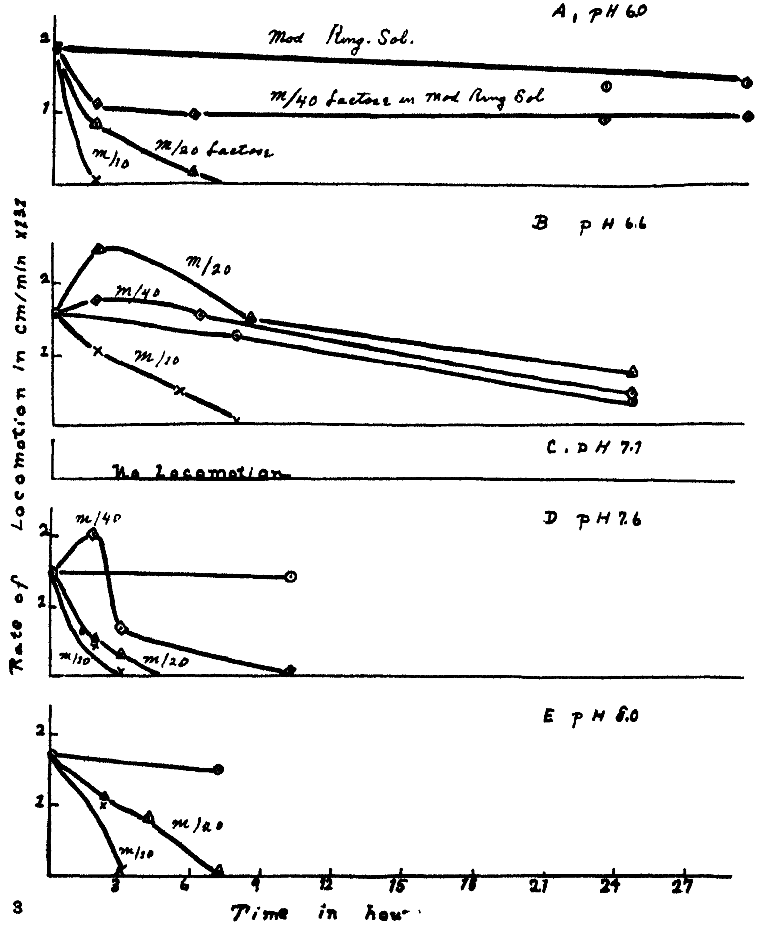

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Fig. 3 Showing, graphically, the relation between the rate of locomotion and concentration of lactose in modified Ringer solution II. The rate of locomotion is plotted as ordinates and the time as abscissas. ⨀, control (modified Ringer solution II); □, M/40 lactose; △, M/20 lactose; ×, M/10 lactose. A, B, C, acid amoebae; C, D, E, alkaline amoebae.

hours, a marked increase in rate of acid amoebae in M/20 lactose at pH 6.6, and of alkaline amoebae in M/40 lactose at pH 7.6. These facts seem to contradict the conclusion that the rate of locomotion is specifically correlated with the water content in Amoeba. This is, however, not necessarily true. In the Ringer solution without lactose the rate of locomotion as indicated in figure 1 is considerably higher for acid amoebae at pH 6.0 than at pH 6.6, and somewhat higher for alkaline amoebae at pH 8.0 than at 7.6. If it is assumed that the water content of acid amoebae at pH 6.0 and of alkaline amoebae at pH 8.0 is optimum and that at 6.6 and 7.6, respectively, it is supra-optimum, resulting in more rapid locomotion at pH 6.0 and 8.0 than at pH 6.6 and 7.6, then the increase in rate of locomotion of acid amoebae at 6.6 and of alkaline amoebae at 7.6 after the addition of lactose is precisely what would be expected, provided the rate is specifically correlated with the water content. If this conclusion is valid, obviously the addition of lactose to the Ringer solution at pH 6.6 and 7.6 should cause an increase in the rate of locomotion, no matter how much or how little is added. The graphs in figure 1 indicate, however, that there was no increase in M/10 lactose at pH 6.6 and in M/10 and M/20 at pH 7.6. This does not, however, demonstrate that there was no increase, for no observations were made until after the amoebae had been in the solution fifteen minutes. It may, therefore, well be that there was an increase in rate in these solutions before the observations were made, and, if this is true, the results support the conclusion that the rate of locomotion in Amoeba is specifically correlated with the water content.

In the modified Ringer solution at pH 7.1 in both acid and alkaline amoebae locomotion ceased before lactose was added and was not resumed upon addition of lactose in any concentration. This can be explained by assuming that the water content of both acid and alkaline amoebae in modified Ringer solution II at pH 7.1 is below optimum and that the addition of lactose consequently augments the unfavorable conditions.

While in any solution amoebae will assume a variety of forms, there is a distinct tendency in each solution to assume some particular shape which might be called the typical form for that solution.

In figure 4 we have presented sketches of the typical form for each of the above concentrations of lactose in modified

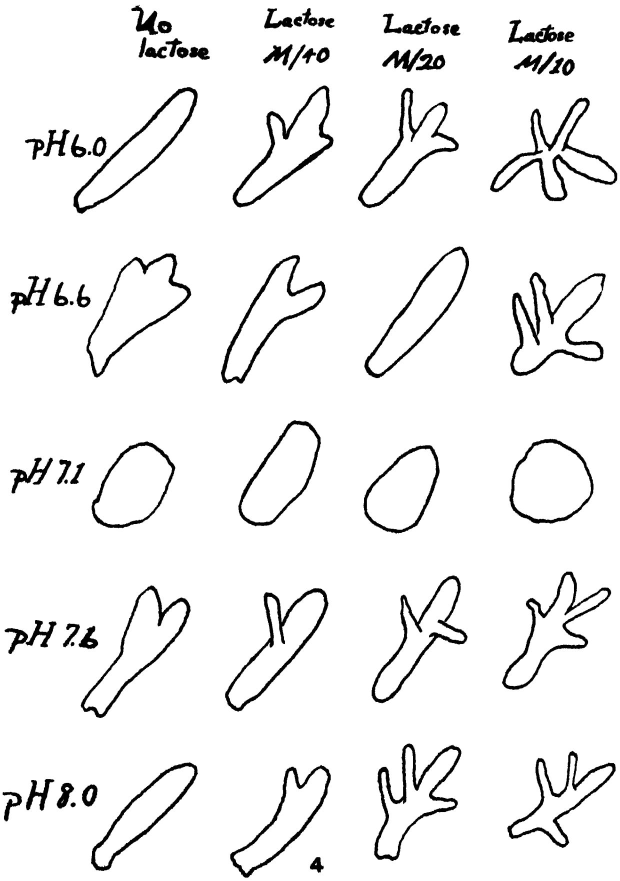

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Fig. 4 Sketches showing diagramatically the relation between the effects of osmotic pressure upon the form of acid and alkaline amoebae and the hydrogen-ion concentration. pH 6.0, 6.6, 7.1, acid amoebae; pH 7.1, 7.6, 8.0, alkaline amoebae.

Ringer solution II of the different hydrogen-ion concentrations. From these sketches it will be seen that in solutions in which high rates occurred, monopodal or nearly monopodal forms prevailed, and that, where low rates occurred, multipodal or rounded forms dominated.

## SUMMARY

1. Form, consistency, rate of growth, rate of multiplication, and rate of locomotion in Amoeba proteus depends upon the hydrogen-ion potential of the solution in which it is immersed. The rate of growth, multiplication, and locomotion are, under certain conditions, maximum at pH 7.6 and, under others, at pH 6.6, and minimum at pH 7.1.
2. Amoebae are usually monopodal in modified Ringer solutions, pH 6.0 and 8.0, and become more and more multipodal and lobose toward neutrality, until they reach pH 7.1, where they become nearly spherical.
3. If amoebae from alkaline cultures, pH 7.6, which have nearly ceased locomotion in modified Ringer solution, pH 7.1, are put into this solution at pH 6.0 or 8.0, locomotion immediately increases. In amoebae from acid cultures, this is true only in hydrogen-ion concentrations below pH 7.1.
4. When amoebae from alkaline cultures, pH 7.6, or acid cultures, pH 6.8, are allowed to come in contact locally with modified Ringer solution of a higher or lower hydrogen-ion concentration, there is an immediate response in the form of rapidly throwing out of a pseudopod in the direction of the contact.
5. The rate of locomotion in Amoeba depends upon the osmotic pressure of the surrounding medium.
6. There is an optimum external osmotic pressure for locomotion in Amoeba which varies with the hydrogen-ion concentration. The optimum external osmotic pressure for locomotion at pH 6.0 or 8.0 is lower than it is at either pH 6.6 or 7.6.
7. The rate of locomotion in Amoeba seems to be dependent upon the water content.

8. It appears that the water content of Amoeba is in some way dependent upon the hydrogen-ion concentration of the surrounding medium.

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# OBSERVATIONS ON THE FAT-BODY IN HYMENOPTERA

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SIX PLATES

AUTHOR'S ABSTRACT

An histological and cytological study of the postembryonic history of the fat-body in Pteronidea ribesi (Scopoli) and Diphadnus appendiculata (Hartig) (both Tenthredinidae) and in Macrocentrus ancylivora Rohwer (Ichneumonidae).

The two principal components of the fat-body are the urate-storing excretory cells and the fat-cells.

The development and behavior of the excretory cells, especially during the metamorphosis, are described, and in Pteronidea their origin is traced to leucocytes which have become associated with the fat-cells.

In the albuminoid inclusions found in the fat-cells two types of substance are distinguished: *a*) a basophile material, of nuclear origin, which appears only during the metamorphosis; *b*) an acidophile material which appears already during the early larval stages in Macrocentrus, but in Pteronidea is formed only during the metamorphosis and in association with the basophile material. It is thus found that albuminoids formed slowly during larval life may exist from the beginning in the form of acidophile spheres, as occurs in Macrocentrus. But those formed rapidly during the metamorphosis, in all the forms studied, are formed in association with a basophile material derived from the nucleus.

The significance of intracellular changes during the metamorphosis is discussed, and the final disposition of the cell inclusions as well as of the fat-cells themselves is described.

## CONTENTS

Introduction .......... 122
The fat-body as an organ of reserve .......... 124
  1. In the non-holometabolic insects .......... 124
  2. In the holometabolic insects .......... 124
Characteristics of the fat-body in the holometabola .......... 125
  1. Characteristics common to all holometabolic insects .......... 125
  2. Comparison of the fat-body in the holometabolic orders .......... 125
    *a.* Cell division .......... 125
    *b.* Albuminoid granules .......... 126
    *c.* The nucleus .......... 126
    *d.* Fate of the larval cells and origin of the imaginal fat-body .......... 127
  3. The excretory function of the fat-body .......... 130
Material and technique .......... 131
Anatomical observations .......... 132
  1. Morphology of the fat-body in Pteronidea and Diphadnus (Tenthredinidae) .......... 132
    *a.* Organization .......... 132
    *b.* Oenocytes .......... 132
    *c.* Leucocyte colonies .......... 133
    *d.* Urate cells .......... 133

2. Development of the fat-cells in Pteronidea and Diphadnus ......... 134
    *a.* In the embryo and larva ......... 134
    *b.* In the mature larva ......... 136
    *c.* In the prepupal instar ......... 137
    *d.* In the spinning prepupa ......... 139
    *e.* In the late prepupa and pupa ......... 140
    *f.* In the imago ......... 142
3. The fat-body in Macrocentrus (Ichneumonidae) ......... 143
    *a.* Larva ......... 143
    *b.* Prepupa ......... 144
    *c.* Pupa ......... 146
    *d.* Late pupa and imago ......... 147
The fat during metamorphosis ......... 148
The urates in the fat-body ......... 149
1. Origin and development of the urate cells in Pteronidea ......... 149
2. Development of the urate cells in Macrocentrus ......... 151
3. Source and significance of the urate concretions ......... 152
4. Urates in non-holometabolic insects ......... 154
The albuminoid inclusions ......... 155
1. The basophile substance ......... 155
2. The acidophile substance ......... 159
3. The fate of the albuminoids ......... 160
4. Other theories concerning cell inclusions ......... 160
    *a.* The granula theory ......... 160
    *b.* The origin of yolk ......... 161
    *c.* Mitochondria ......... 162
Interpretation of intracellular changes ......... 164
1. Fat and glycogen ......... 164
2. The albuminoids ......... 164
3. Significance of the albuminoids ......... 165
Acknowledgment ......... 167
Summary ......... 168
Literature cited ......... 170
Explanation of plates ......... 174

## INTRODUCTION

In the mature larva of holometabolic insects the fat-body is, as regards bulk, the dominant tissue in the body. The larva which has thus become largely an accumulation of reserve materials is not merely an immature form of the imago, but is a form which has acquired, caenogenetically, a number of adaptations not present in the imago. These newly acquired organs, which the larva possesses in addition to the imaginal organs that may be already developed, or present in the form of anlagen, adapt the larva to a life under entirely different conditions from those encountered by the imago. Being purely larval organs, they must be considered as having been acquired by the larva independently of imaginal structures. The larva has in this way developed into a very efficient accumulator of reserves which are stored in a readily convertible form, and it is at the expense of these reserves that the imaginal organs, whose development has been suppressed during larval life, rapidly develop during the resting stage which intervenes before the emergence of the imago.

These reserves are present partly in the form of variously specialized larval tissues such as the larval muscles, hypodermis, alimentary epithelium, malpighian tubes, glands and nerve cells, all of which are more or less completely replaced during the metamorphosis by newly formed imaginal tissues. However, the greatest mass of reserve material is represented by the fat-body, in the cells of which accumulate large amounts of fat and other reserves that are subsequently transformed within the cells and used in the histogenesis of the imaginal organs. The processes involved and the mechanism of the transformation of the larva into the imago are as yet imperfectly understood, so that, in spite of many arduous investigations upon the nature of the internal changes involved, the information on hand is insufficient to yield a generalized comparative picture of these changes for all the holometabolic insects. Certain phases of this transformation have, however, been fairly intensively investigated, and of the tissues involved the fat-body has probably been more thoroughly studied than any other. This interest was due in a large measure to the obvious availability of the fat-cells as objects for histological and cytological investigations. The large size of the cells themselves and of their inclusions of fat and albuminoids seemed to indicate that in these cells, if in any, it should not be difficult to study the cytological changes associated with definite types of metabolic activity, especially the extensive changes taking place during the sudden and intensive activity accompanying the period of transformation from larva to imago. Histologically, too, the study of the fat-body as to its development in the larva, its diversities of structure and behavior in the various insect groups, and the fate of its constituent cells during metamorphosis has offered interesting problems. Brief historical accounts of the literature bearing on these investigations may be found in a number of the more recent papers (Anglas, '00; Perez, '02, '11; Kreuscher, '22). There will be outlined here a comparative account of some of the characteristic features of the fat-body as occurring in the different orders of holometabolic insects.

## THE FAT-BODY AS AN ORGAN OF RESERVE

Reserve food materials, stored in the living cells, are met with in animals of all groups, usually in the form of fats, albuminoids, and glycogen. In insects, reserves are commonly found in the form of fats and albuminoids. Glycogen has also been reported by Herrmann ('21), Nakahara ('17), and others.

### *1. In the non-holometabolic insects*

In those insects which do not undergo transformation the fat-cells fulfill their normal function as reservoirs for reserve foods, containing within their cytoplasm vacuoles filled with fat, the amount varying with the condition of nutrition in the insect. In addition to the fat-droplets, there may be present granules representing albuminoid reserves. These granules are not always confined to the fat-cells, but, as described in Collembola (Philiptschenko, '06), may also be present in the adjacent hypodermal cells.

### *2. In the holometabolic insects*

In the holometabolic insects greater interest attaches to the fat-body. When in these insects the larva has attained its maximum size and has entered the prepupal phase, the changes initiated involve every tissue, and, as regards the fat-body, the activities associated with this transformation do not cease until some time after the imago has emerged from its pupal skin.

During larval life the fat-cells increase in size and become filled with fat-droplets and in some forms also with albuminoid granules. In the imago a variety of conditions may be encountered according to the particular insect studied: the larval fat-cells have disappeared either entirely or in part and the fat-body of the imago is composed either of persisting larval cells or of newly formed imaginal fat-cells or even of a combination of both types. The changes going on in the larval fat-cells during metamorphosis as well as the mode of disintegration of those cells which do not persist in the imago vary considerably in the different orders and even within an order.

## CHARACTERISTICS OF THE FAT-BODY IN THE HOLOMETABOLA

### *1. Characteristics common to all holometabolic insects*

There are certain characteristics common to the fat-body of all the holometabolic insects: 1) The fat-body cells contain fat-vacuoles at all stages in the life-history, except in certain embryos and young larvae. 2) Albuminoid granules are present, at least during metamorphosis. 3) Urates are accumulated in the fat-body, at least during metamorphosis. These urates may be stored either in the fat-cells themselves or in special excretory cells intimately associated with the fat-cells. 4) At least some of the fat-cells persist and continue to function in the imago.

### *2. Comparison of the fat-body in the holometabolic orders*

Upon examining the history of the fat-cells in the different orders we find that a variety of conditions has been reported.

*a. Cell division.* First, as to mitotic multiplication of fat-cells during larval life, we find that such does occur in the Coleoptera (Poyarkoff, '10; Berlese, '99–'01; Kreuscher, '22) and in the Lepidoptera (Hufnagel, '18; Berlese, '99–'01) usually at the time of a larval molt, while no multiplication at all has been reported in the larva of Diptera and Hymenoptera (Perez, '10, '12, and earlier authors), except in recently hatched honey-bee larvae (Schnelle, '23).

*b. Albuminoid granules.* Then, as to the times at which albuminoid granules are present in the fat-cells, we find that often these are present only during the period of transformation, making their appearance after the last larval molt, rapidly increasing in quantity during the earlier part of transformation and then again disappearing in the late pupal phase and during the beginning of imaginal life. In all except the Diptera, however, are found forms in which the granules already appear during the first half of larval life. Instances recorded are, in Lepidoptera: Pieris (Berlese, '99–'01); Nakahara, '17); in Coleoptera: Aphodius, Lampyris, and Sitodrepa (Berlese, '99–'01); in Hymenoptera: Cynips, Tropinoma (Berlese, '99–'01), Nasonia (Tiegs, '22) and Formica (Perez, '02). Berlese, in respect to the Hymenoptera and Lepidoptera, also points out that in larvae which spin a large amount of silk the albuminoids appear later than in other forms. Then, too, it seems that in Dytiscus, according to Kreuscher ('22), granules may also occur in the imago as a normal reserve, and not merely as remains from the pupal stage.

*c. The nucleus.* In respect to the nucleus of the fat-cells, it is interesting to note that in the Coleoptera (Berlese, '01; Kreuscher, '22), in the Lepidoptera (Berlese, '99–'01; Samson, '08; Hollande, '14; Hufnagel, '18), and in the Diptera (Perez, '10, and earlier authors) this structure in nearly every instance remains more or less spherical or ovoid, while in the Hymenoptera, directly after the last larval molt, the nucleus becomes greatly deformed, elongated, and with linear branches extending through the cell, in Monodontomerus even becoming fragmented (Berlese, '99–'01). It appears, too, that during this change in form the nuclear membrane may break down, more or less, temporarily. In the imago the nucleus retains this bizarre form acquired during metamorphosis (Karawaiew, '98; Koschevnikow, '00; Berlese, '99–'01; Perez, '10, '12; Bishop, '22; Schnelle, '23). However, exceptions exist to the above general rule; in Anthonomus pomorum (Col., Ryncoph.) Henneguy ('04) finds the nucleus branched and ramified, in Pieris (Lepid.) Nakahara ('17) describes amitotic division of the nucleus, and Berlese ('99–'01) finds that in Cynips (Hym.) the nucleus always remains rather spherical.

*d. Fate of the larval cells and origin of the imaginal fat-body.* We again find differences in regard to the fate of the larval fat-cells and in regard to the origin of the imaginal fat-body as reported by investigators in the several holometabolic orders.

In the Coleoptera, according to Berlese ('99–'01), Poyarkoff ('10), and Kreuscher ('22), the majority of the fat-cells survive metamorphosis and with little change form the imaginal fat-body.

In the Lepidoptera the larval fat-cells which during metamorphosis had become isolated and free from one another are, in the imago, again united into groups surrounded by an enclosing membrane, and thus come to form the fat-body of the imago (Berlese, '99–'01; Hufnagel, '18). Hufnagel believes that a phagocytosis of some of the fat-cells must take place during the transformation in Hyponomeuta, since there are fewer fat-cells in the imago than were present in the larva. However, there is no convincing evidence presented and it is more probable that the cells which disappear simply undergo dissolution after having yielded up their inclusions to the blood.

In the Diptera we find that the earlier investigators (Weismann, '64; Auerbach, '74; Ganin, '75; Künckel d'Herculais, '75; Viallanes, '82; Kowalewsky, '85; van Rees, '88) described a traumatic disintegration of fat-cells during transformation. Of the authors mentioned, only Weismann, Ganin, and Künckel failed to misinterpret their observations. More recently, both Mercier ('06), Perez ('10), and Snodgrass ('24) have again observed traumatic histolysis of the fat-cells; Mercier particularly, employing carmine injection in order to identify the leucocytes, finds that these will invade only those fat-cells which have already ruptured spontaneously. Berlese ('99–'01) and Enriquez ('01, '02) also agree that the phagocytes take no part in the actual breaking down of the cells. Kowalewsky ('85), on the other hand, observed phagocytosis in vivo of fat-cells in the head, and Perez ('10), in addition to observing a traumatic breaking down followed by phagocytosis, also describes the phagocytosis of intact fat-cells which in no way show any sign of degeneration. Most of the larval fat-cells, according to Perez, go over into the imago, in which they gradually become smaller as their reserves are used up and are surrounded by leucocytes which gather about them. There is, in addition, a true imaginal fat-body, formed at the end of the pupal period from hitherto undifferentiated embryonic cells. Berlese also describes an imaginal fat-tissue in the higher Diptera, as formed by the mitotic proliferation of the nuclei of the Kernchenkugeln—a theory amply criticised by Perez. In the less evolved Diptera (Nematocera), according to Berlese, the larval fat-body is preserved in the imago. Kellogg ('01) reports the absence of phagocytosis in the Tipulid, Holorusia, but in Bepharocera, another of the Nematocera, he does observe phagocytic destruction.

Finally, as to the fate of the fat-cells in the Hymenoptera, we find such diversity of views that it is often difficult to decide whether the described differences are due to disparity in interpretation, to faulty technique, or to actual diversity in the material. The authors, however, agree that the fat-body of the imago is constituted of larval fat-cells which have undergone a rearrangement in the pupa. Probably not all the fat-cells go over into the imago, as Berlese thought, but only a part of them.

Three methods of disposal have been described for those cells which disappear during transformation: 1) phagocytosis, 2) traumatic disintegration of the cells, 3) resorption of cell contents accompanied by diminution in size of the cell and followed by phagocytosis of the residue or the solution of the residuum in the body fluid.

Perez describes typical phagocytosis, occurring at an early stage of transformation in Polistes, as removing a large number of the fat-cells, especially those situated in the thorax.

Perez also describes the phagocytosis of the remains of fat-cells which have persisted into the late pupa or early imago and whose inclusions have been gradually resorbed. Such typical phagocytosis has not been recorded by other authors (Koschevnikow, '00; Berlese, '99–'01; Terre, '00; Anglas, '02; Bishop, '22; Schnelle, '23). Karawaiew ('98) described what he thought to be phagocytosis of fat-cells, but from his accurate description of the phagocytes, which are attached to the exterior of the fat-cells, it is obvious that he was dealing with the urate cells.

Traumatic disruption of larval fat-cells during transformation is described, especially as occurring in the bee, by Koschevnikow ('00), Bishop ('22), Schnelle ('23), and in the wasp by Anglas ('02). Of the cells in the wasp, Anglas states that only some of these display traumatism, while others degenerate without breaking up. According to the former authors, the fat-cells, after having attained their maximum size and being filled with inclusions of fat and albuminoids and having become free from each other, rupture and spill their contents into the body cavity so that the body liquid becomes a kind of purée composed of the blood plasma thickened with albuminoid granules, fat-droplets, and nuclei, all derived from the disintegrated fat-cells. Such disruption of the fat-cells has not been described by Berlese nor by Perez. Perez ('12) finds no traumatism at all in Polistes and believes that such observations must be the result of poor technique.

Degeneration without phagocytosis is described by Terre ('00) in the bee and by Anglas ('02) and Perez ('10, '12) in the wasp. Anglas observed that the cells grow smaller and finally disintegrate, as he believes, due to the activity of enzymes secreted by leucocytes which are found in the immediate vicinity of such cells—a phenomenon he calls lyocytosis.

### 3. *The excretory function of the fat-body*

In regard to the urate-storing function of the fat-body, one again finds wide differences both as to the times in the life-history during which urates may be found and as to whether they are stored in the fat-cells themselves or in special 'excretory cells' associated with the fat-cells.

In Collembola, according to Philiptschenko ('06) and others, in Orthoptera (s.l.), according to Cuenot ('95), and in the Hymenoptera (with the exception of Monodontomerus, as recorded by Berlese), according to Berlese ('99–'01), Anglas ('02), Henneguy ('04), Perez ('12), Schnelle ('23), Bledowsky and Krainska ('26), and earlier authors, special cells are found in the fat-body which are entirely filled with concrements of urate.

In the Coleoptera, according to Berlese ('99–'01), Poyarkoff ('10), and Kreuscher ('22), and in the Lepidoptera, according to Berlese ('99–'01), Hollande ('14), and Samson ('08), on the other hand, the urates are stored in the fat-cells themselves.

In the Diptera Berlese ('99–'01) described in the fat-cells of pupae the occurrence of small spheres containing deeply staining (haemalum) granules, interpreted as reserves in the process of transformation, and named them 'pseudonuclei.' These same objects were again observed in Calliphora by Perez ('10) who, relying in part upon the tests carried out by Marchal ('89) and in part upon the fact that these granules stain with haemalum as do the urates which he himself observed in the ant, interprets them, however, as urate granules. If the 'pseudonuclei' of the dipterous cells shall really be found to contain urates, then we should have in the Diptera a special kind of urate-storing fat-cell in which the urate crystals do not appear in separate vacuoles as in Coleoptera and Lepidoptera, but within the albuminoid inclusions. The location as well as the small size of these granules does not readily permit the application to them of the murexid test which is really necessary to determine their nature.

## MATERIAL AND TECHNIQUE

The species employed in this study were two Tenthredinids, Pteronidea ribesi (Scopoli), and Diphadnus appendiculatus (Hartig), both of which feed on the leaves of the cultivated gooseberry, and an ichneumonid, Macrocentrus ancylivora Rohwer, a parasite upon larvae of Ancylis comptana Froehl., the strawberry leaf roller, and of Laspeyresia molesta Busck, the oriental peach moth. Serial sections of entire insects of all stages were prepared in addition to smears and dissections. The serial sections of Macrocentrus larvae were prepared from material bred from Ancylis, while the sections of postlarval stages (prepupa, pupa, imago) and all the smear preparations of Macrocentrus were made from individuals bred from Laspeyresia. The fixatives employed in preparing the sectioned material were mainly Allen's B15, Flemming's and Champy's fluids.

The insects were first dipped in chloroform and then immediately dropped into the fixative. The chloroform aids greatly in securing penetration of the fixative through the chitin, and without its use fixation of Macrocentrus larvae is hardly possible except with Carnoy's fluid. Allen's B15 was warmed to ca. 80°C. and material fixed in Flemming or Champy was cut open to secure sufficient penetration of the osmic acid for fixation of the fat-globules. Recalcitrant specimens which refuse to fix in fifteen minutes are discarded.

In preparing the serial sections the usual paraffin method was used, clearing in aniline oil from 70 per cent alcohol and transferring through chloroform gradually into paraffin. Sections were cut at 6 to 8 μ and stained with Heidenhain's iron-haematoxylin with or without counterstains or with haemalum (newer formula) with counterstains, such as eosin, orange G, van Gieson's, acid-fuchsin, and lichtgrün. Some slides were stained with Mallory's connective-tissue stain.

Smear preparations of the fat-cells were fixed with 4 per cent formalin and stained with haemalum and sudan III or alkanin and mounted in glycerin jelly, or stained with haemalum and a counterstain and mounted in balsam.

For investigating the development of the urate cells, material was fixed in absolute alcohol, since the urate crystals are water-soluble, carried directly into xylol and paraffin and stained in alcoholic solutions.

The murexid test was also applied to urates obtained from the fat-body of the pupa and the imaginal excrement of Pteronidea and from the larva, pupa, intestinal content, and imaginal excreta of Macrocentrus. In every case a beautiful purple color was obtained.

## ANATOMICAL OBSERVATIONS

### 1. *Morphology of the fat-body in Pteronidea and Diphadnus*

*a. Organization.* The morphology of the fat-body in the two sawflies studied corresponds to the descriptions of this organ given by Berlese ('99–'01) and Wilke ('21). In the larva two major elements are recognized, the central fat-body and the peripheral fat-body.

The central fat-body is a cylindrical structure composed of fat-cells connected by threads or 'cell bridges,' which are prolongations of the cell membranes, forming a loose network enveloping the alimentary canal and extending through the body from the first thoracic segment to the posterior end of the abdomen. Integral parts of this fat-body are the two ducts of the spinning gland and the acini of this gland which are attached to each duct in two rows. The acini superficially resemble the fat-cells both as to cell size and nuclear structure in the young larva.

The peripheral fat-body is composed of the remaining fat-cells which lie nearer the body wall, distal to the pericardial septum, the ventral septum, and the inner layers of muscles. These cells are not so uniformly distributed as are the central fat-cells, but are more numerous in the ventrolateral lobes of the body segments.

*b. Oenocytes.* Associated with the peripheral fat-cells and closely resembling them in the structure of both nucleus and cytosome, so that in sectioned larval material their likeness to the former is almost deceptive, are the oenocytes. These cells, to which, in the absence of any positive knowledge, a secretory function of unknown import is generally ascribed, are usually large rounded cells with dense homogeneous cytoplasm. In this form we find them also in the embryo and in the imago of Pteronidea, while in the larva the cytoplasm is very coarsely granular and vacuolated.

*c. Leucocyte colonies.* Leucocyte colonies are also found distributed throughout the peripheral fat-body, especially in the dorsal region and in the ventrolateral segment lobes. In the very youngest larvae one or two of these leucocytes are attached to many of the fat-cells (fig. 3). Mitotic multiplication goes on throughout larval life, so that extensive cell masses are formed, attached to single fat-cells or extending between several neighboring ones (figs. 9, 12). Individual cells are continually set free from these colonies during larval life, and the free leucocytes found in the blood closely resemble those in the colonies except that they have acquired a more extensive cytoplasm.

These blood-cell groups were first described by Schaeffer ('89) in Lyda (Tenth.) and in the thorax only of Hyponomeuta (Lepid.) where they occur at the bases of the wing rudiments. Hufnagel ('18) states that she finds them also in the abdomen in Hyponomeuta and considers them the origin of both the free leucocytes in the blood and the fat-cells. In another lepidopterous larva, Zerynthia, Wegener ('23) describes blood cells originating from the hypodermis of the dorsum. In Pteronidea there is no transformation of leucocytes into fat-cells. The occurrence of such colonies is apparently rare in insects and has been described only in the groups above mentioned. The cells evidently correspond to the 'small amoebocytes' of Cuenot and to the 'chromophile leucocytes' of Muttkowski. After liberation, they transform by addition of cytoplasm to the 'large amoebocytes' of authors. During transformation the leucocyte colonies disintegrate and the cells are released into the body cavity.

*d. Urate cells.* Another constituent of the fat-body is the urate cells which in the sawflies are confined to the central fat-body.

### 2. *Development of the fat-cells in Pteronidea and Diphadnus*

*a. In the embryo and larva.* In the late embryo of Pteronidea the cells of the fat-body are already all formed and undergo no further multiplication during the growth and development of the larva, their later history has to do only with an increase in size and with differentiations of internal structure.

In the embryo the central fat-cells are found to be ca. 5 µ in diameter and with a spherical nucleus 3 µ in diameter (fig. 2). The nucleus is filled with lighter-staining granular substance and usually one dark chromatic mass. The cytoplasm is dense and homogeneous and contains several small fat-vacuoles. The peripheral fat-cells (fig. 1) are somewhat larger (6 to 7 µ). The nucleus (3 to 4 µ) is again round and finely granular, with one large irregular chromatic mass. The position of the nucleus in the cell is generally eccentric, due to the presence in the cytosome of a large fat-vacuole in the otherwise homogeneous cytoplasm.

In feeding larvae of the first instar the central fat-cells have increased to 10 µ in diameter (fig. 4). The centrally situated spherical nucleus contains normally two irregular chromatic masses in a matrix of lighter chromatic material. The heavily staining cytoplasm, irregularly flocculent in appearance, is differentiated into an irregular zone of very dense material about the nucleus. Fat-vacuoles are not always recognizable in these cells and, especially in the thorax, one finds many cells with apparently no fat-vacuoles, while in the abdomen they are usually clearly seen. Cells without spherical vacuoles usually have irregularly outlined vacuoles containing a lightly staining, smooth, homogeneous substance (fig. 4).

The peripheral fat-cells of this stage are somewhat larger than the central fat-cells. They measure ca. 16 µ and their nucleus has a diameter of 6 µ. The structural details of both nucleus and cytosome resemble closely those of the central fat-body (figs. 3, 5, 6).

During the second and third instars the cells continue to grow so that in the third instar we find that their diameter has increased to 30 μ, their nucleus to 10 μ (figs. 7 to 12). The nucleus has retained its spherical or ovoid form, but its membrane is now rather thick and the chromatic material has broken up into numerous small bodies. The cytoplasm is granular and the dense perinuclear cytoplasm may still be in evidence, and, in the peripheral fat-cells, is seen to extend out between the vacuoles to the cell membrane (figs. 9, 10, 12). In the central cells this dense material may be scattered in little islets distant from the nucleus (figs. 7, 11).

Fat-vacuoles are present, occupying at least half the volume of the cytosome in the peripheral cells, while in the central cells they may be obscure or apparently absent. In cells of the latter group one again finds irregular vacuolated areas filled with homogeneous achromatic material, and it is these cells which have the most deeply staining cytoplasm—a condition characteristic of some individuals in which the central fat-cells are more retarded in their development by comparison with the peripheral cells (compare figs. 7 and 10, from the same larva; also 11 and 12). The fat solvents used in the preparation of sectioned material rarely leave the vacuoles of this stage entirely clear, some lightly staining substance usually remains either in the form of sparsely scattered granular material (fig. 9) or in the form of smooth-lumped masses (figs. 8, 10).

In the fourth instar the cells have attained a size of 40 to 50 μ. In a central fat-cell (fig. 13) we find conditions similar to those in the preceding instar, but the nucleus is surrounded by a clearer zone similar to the vacuolated areas out in the cytoplasm. A peripheral fat-cell (fig. 14) shows the dark perinuclear material with radiating arms extending out to the cell membrane, and we note, in addition to the small clear fat-vacuoles, two large vacuole-like areas filled with a light granular material. Figure 15, as contrasted with figure 13, illlustrates a central fat-cell in which the fat is in the form of definite clear vacuoles and in which the cytoplasm is much less dense.

During the last larval instar (figs. 16 to 18) the fat-cells may range in size between 70 and 140 μ, rapid growth taking place at this time so that the cells attain almost their maximum size. The nucleus loses its spherical form, becoming indented, and at the beginning of this instar already has spinous projections extending outward. The fat-vacuoles are distinct and are found at the periphery of the cell, with the exception of a few smaller ones which may be nearer the nucleus. The amount of fat present in the peripheral cells always exceeds that in the central cells (compare figs. 17 and 16). Vacuolated areas present just outside the nuclear membrane appear to indicate that the substance contained in them has been extruded from the nucleus, the membrane of which is at this time very delicate or in some places entirely absent.

*b. Mature larva.* In a fully mature larva (fig. 18) the nucleus is still more deformed and a number of elongated arms radiate out from the principal nuclear mass and extend almost to the peripheral region of the cell. The investiture of the nucleus is now a thick, dense, achromatic membrane which also extends outward from the ends of the nuclear arms, branching and radiating into the cytosome, and making contact at numerous points with the cell membrane, at whose surface the prolongations of this network form a continuous layer. This extensive cytoplasmic network connecting the nuclear envelope with the cell membrane appears to act as a supporting structure. The network itself does not stain except with cytoplasmic dyes, but with Flemming's tricolor stain it becomes an intense blue, the stain being confined to the surfaces and to minute granular material on these surfaces. Under some conditions, iron-haematoxylin will also blacken this network. In the interstices of this network there is a light granular cytoplasm which stains rather strongly with acid dyes; centrally, the portion partly surrounded by the arms of the nucleus consists of a very light open reticulum.

If we compare this cell (fig. 18) with an earlier stage (fig. 14), it appears that the more chromatic cytoplasm which in the earlier stages envelops the nucleus and extends outward has in the older cell become condensed into a homogeneous meshwork. This mesh serves to anchor the nucleus to the cell membrane, and as the cell grows, nuclear growth in the meantime not keeping pace with cell growth, the radiating strands tend to keep those points of the nucleus to which they are attached nearer the cell membrane so that the nucleus must necessarily become stretched out into the branched form it comes to have. Figures 16 and 17 are from Flemming-fixed material of an earlier stage and these strands do not appear, yet the sharply drawn-out projections on the nucleus seem to indicate their existence.

Increase in size of the fat-cells during the last larval instar takes place by the addition of new cytoplasm in the region next the nucleus, as evidenced by the exclusively peripheral position of the fat-vacuoles, which are older structures, and by the appearance about the nucleus of the large vacuole-like masses of light, reticular cytoplasm differing from the denser peripheral protoplasm.

After the last larval molt we have to do with the prepupal instar which at first resembles the grown larva except for the absence of any pigment in the chitin and of the spines which are characteristic of the larva. After shedding the larval skin, the prepupa rests for several hours, then burrows into the ground (Pteronidea) or remains on the stems of the food plant (Diphadnus), spins a cocoon, in about nine days (in the early spring generation) molts to the pupa, and in another three or four days emerges as an imago.

*c. Prepupal instar.* In a prepupa which has just shed its larval skin, changes occur rapidly in the fat-cells. The nucleus retains its curious form, but the heavy nuclear sheath becomes wide, loose, and reticular in some places, especially in its distal parts, and lying in and on this structure and on the cytoplasmic network as far as the cell periphery are found numbers of small globules, the beginnings of the albuminoid inclusions (figs. 19 to 21).

These small albuminoid spheres, at first sparsely scattered through the cell, are granular in structure, staining lightly with haemalum, and when acid stains are added, accepting these also and becoming much darker (fig. 21). When iron-haematoxylin is used, the spheres appear to be composed of an achromatic matrix containing minute chromatic granules (figs. 19, 20). The cytoplasm, especially that part lying near the nucleus, has a strong affinity for acid dyes, but does not stain at all with haematoxylin.

This sudden appearance of albuminoid granules in the cells indicates a period of great metabolic activity in the cytoplasm, as the result of which cytoplasm, and probably fat also, is transformed into an albuminoid substance. Since cell activities generally are dependent upon stimuli, probably in the form of enzymes, emanating from the nucleus, it is not surprising to find that extensive nuclear changes occur at this time. In fact, we have seen (fig. 18) that a great change in nuclear form has already preceded the appearance of the albuminoids, for at this early stage, although the nucleus is highly deformed and ramified, there are no staining granules in the cytoplasm, but only a very few achromatic hyaline grains which are very minute and whose significance is not clear. Then in figures 19 to 21, where the albuminoids are seen in the initial stages of formation, we find that the membrane of the nucleus has undergone a change, having become in some places very thin or even diffuse, but in most parts retaining its original structure, and that the volume of the nucleus, as well as the number of chromatic granules contained in it, has been greatly reduced.

It does not seem, however, that the large number of albuminoid spheres in the cytosome can be accounted for by supposing that they have originated from chromatic nuclear granules which have emigrated from the nucleus. Such an origin is maintained for the albuminoids in the honey-bee by Bishop ('22, '23).

If the cells under consideration behaved according to Bishop's theory, we should expect to find basophile granules appearing in the cytosome as soon as the nucleus becomes ramified, but such is not the case (fig. 18). Again, the albuminoid granules when they first appear are found throughout the cytosome (figs. 19 to 21), and they do not at all resemble the chromatic granules of the nucleus. Without a knowledge of this early condition of the albuminoids, however, an examination of a later stage (fig. 22) when stained with iron-haematoxylin might at first suggest that here, too, the albuminoids are formed in the manner described by Bishop, for we find black granules in the cytoplasm resembling in size and color the granules in the nucleus, although they are entirely different, as indicated by their affinities for other stains.

At the time the prepupa is beginning to spin its cocoon, a few hours after the last larval molt, we find (fig. 21) that the albuminoids have increased in number and in size, but have remained similar in appearance.

*d. Spinning prepupa.* In a prepupa which has begun spinning (figs. 22 to 24) the cytoplasm is already well filled with numerous albuminoid inclusions of different types, the nucleus is somewhat simpler and the nuclear granules are in a more stable, spherical form. A heavy nuclear membrane is present, but is not uniform, in some parts being still thin or diffuse.

At this time, in material fixed in Allen's B15 and stained with iron-haematoxylin, there may be distinguished in the fat-cells three types of albuminoid inclusions, viz., 1) granular spheres, 2) smooth, achromatic spheres, 3) solid black spheres. There are also present some which may be considered as transitional types, between the granular and the achromatic spheres and between the achromatic and the black spheres.

Although all these different types of albuminoids may be present within a single cell, their relative abundance varies in different regions of the body. Estimates made of the relative numbers present in the cells of different regions showed that, as regards the central fat-body, *a*) the granular albuminoid spheres are relatively scarce in the thorax and gradually increase in numbers caudad; *b*) the achromatic spheres predominate in the thorax and gradually become less abundant, so that only a few are present in the cells at the posterior end of the abdomen; *c*) the black staining spheres are fewer in the thorax and very numerous in the abdomen; *d*) spheres which, according to their staining reaction, are transitional between the achromatic and the black albuminoid spheres are most numerous in the thorax.

In the peripheral fat-body conditions are somewhat similar, except that there are no black granules in the thorax (fig. 23) and comparatively few in the abdomen and that the granular spheres are much more numerous throughout than they are in the central fat-body. In the posterior end of the abdomen are found many transitions between achromatic spheres and the granular type; there are many of the former which contain a few, or often a peripheral ring of minute black granules.

*e. Late prepupa and pupa.* In the late prepupa as well as in the pupa and early imago, using the same technique, it is found that practically all the albuminoids are in the form of spheres which stain intensely with iron haematoxylin (fig. 25). In the central fat-body at this time nearly all of these spheres are refracting bodies which take a translucent black stain, while in the peripheral fat-body they are not refracting, but are more densely black and opaque. In addition, there are present in all cells, especially in the peripheral cells, some granular spheres.

From these observations, made upon material fixed in warm B15 and stained with iron-haematoxylin, it would appear that the black-staining spheres are formed from achromatic spheres which in turn have either arisen independently or, more probably, have developed out of smaller, minutely granular spheres.

In material fixed in absolute alcohol or in Champy's fluid (an osmic mixture without acetic acid) the albuminoid spheres are different in appearance. Figures 26 and 27 are cells from prepupae which had completed spinning; the former is from a specimen fixed in alcohol and stained with haemalum and orange G, the latter from one fixed in Champy's fluid and stained with iron-haematoxylin.

Adjacent to the nucleus are seen numerous smaller spheres which are slightly basic and granular. They evidently represent bodies more recently formed as the result of transferral to the cytosome of substances originally in the nucleus. For it is noted that the nucleus is now more reduced in volume than in the preceding stage; it is very much flattened and contains but little chromatic material. The cytoplasm in the vicinity of the nucleus has a strong affinity for acid dyes.

More remote from the nucleus the albuminoid spheres consist of a hollow shell of very lightly basophile material enclosing a vacuole-like space more or less filled by an intensely acidophile globule. The exterior shell is often more or less crumpled, as if the included globule or some other substance in the living condition completely filled the shell and that this material has been partly dissolved out during preparation. Some of the spheres are entirely hollowed and contain no acidophile material within. These latter resemble closely the fully developed albuminoid spheres, as described in the bee by Bishop.

Between the small granular spheres near the nucleus and the more peripherally located larger spheres all transitions are present, indicating the development of the latter out of the former.

Comparing the observations made on material prepared by the different methods, it is apparent that the spheres which, when fixed with alcohol or Champy's fluid, are partly hollowed, partly more or less filled with an acidophile substance, fix completely and uniformly as solid objects, with warm B15 and react variously, as seen in figures 22 to 24, to iron-haematoxylin, while with haemalum and orange G they are found to be uniformly acidophile, except for an external layer which is slightly basic.

These observations indicate that in Pteronidea the mature albuminoid spheres in the fat-cells are formed from the smaller spheres which are somewhat basophile and granular and which appear throughout the cytosome at an early stage in the prepupa (figs. 19 to 21) and confined to the vicinity of the nucleus in a somewhat later stage (figs. 26, 27). The small spheres grow by the accumulation within their interior of an acidophile substance, while the original granular material remains on the surface of the growing sphere and becomes less and less stainable with basic dyes.

During the early part of prepupal life, while the body is still larva-like in exterior form, in its movements and in its responses by movement to stimulation of the thoracic legs or of the mandibles, the fat-body cells also maintain the arrangement characteristic of the larva. As the form of the prepupa changes, the abdomen becoming shorter and straight and the vague outlines of the future pupal form becoming visible, the fat-body becomes disintegrated so that the individual cells are isolated from each other and lie free in the body cavity—a condition which allows the fat-body to adjust itself to the form of the developing pupa. This condition of the fat-cells is best seen by cutting open the insect and allowing the fat-cells to flow out. At this period the cell membrane is very delicate and the cells are fragile and rupture easily. In the insect, however, the cells always remain intact and very soon the individual cells associate together in small groups of three or four cells and also into strings and layers (fig. 29), in which form they are present in the pupa and in the young imago.

*f. Imago.* As the imaginal organs are being formed during the pupal instar and as the activities of the insect and the development of the gonads continue during early imaginal life, the fat-cells decrease in size, and many of them disappear entirely. The albuminoids gradually disintegrate and dissolve leaving the fat-droplets as the only remaining inclusions in the active imago. Figures 30 and 31 illustrate two groups of fat-cells from the abdomen of a young imago. In the first group are cells with very large fat-vacuoles together with some albuminoids. In the second group (fig. 31) are cells of more normal habitus which would continue to function as imaginal fat-cells. Figure 28 represents a cell from the thorax of a young imago and shows the albuminoid spheres in the process of dissolution. A few of the spheres are still present, others are disintegrating and are very slightly stained, their position being indicated by the form of the surrounding cytoplasm which now stains intensely black with iron-haematoxylin. In some parts of the cell there is no trace at all of the albuminoids, but only a homogeneous cytoplasm. As the albuminoids lose their affinity for iron-haematoxylin, the cytoplasm acquires such an affinity, indicating that substances from the albuminoid spheres have passed into the cytoplasm.

Not all of the larval fat-cells, however, survive transformation. Many, especially those situated in the thorax, disappear entirely after having resorbed their inclusions. They become reduced in size and finally fragment and go into solution in the blood. There is no indication that phagocytes play any part in the removal of these cells.

### 3. *The fat-body in macrocentrus*

*a. Larva.* In Macrocentrus larvae we can again distinguish a central and a peripheral fat-body. The central fat-body, which is the more extensive, occurs in the form of lobed sheets or masses one or two layers thick, in which the individual cells are clearly distinguishable. Intercalated throughout the central fat-body of the abdomen are the urate cells which during the first larval instar already contain sphaerocrystals of urate.

During the first instar the fat-cells average 8 μ in diameter. The nucleus at this time resembles that of the corresponding stage in Pteronidea, and contains, in addition to the granular material, from one to four, usually two, chromatic masses (fig. 32). There is present usually only a single fat-vacuole, the size of which leads to the eccentric position of the nucleus.

In the second instar the cells of the peripheral fat-body (fig. 33) have grown less rapidly than the central cells (fig. 34). In both, the chromatic material of the nucleus is divided into numerous granules—a condition already noted in the second instar of Pteronidea. It is noted that the cytoplasm in these cells is always rather uniformly granular and does not have a denser perinuclear moiety as was observed in Pteronidea.

Appearing first in the second instar are small albuminoid inclusions in the cytoplasm, occurring either isolated or in groups (fig. 34). These granules stain deeply with iron haematoxylin, with acid dyes, such as eosin, orange G, and acid fuchsin, but not at all with haemalum. When they first appear the granules are found only in the abdomen, but toward the end of the second instar are found throughout the thorax and abdomen in the cells of the central fat-body and in a few of the peripheral fat-cells.

In the third instar, which is the last larval instar, the granules have attained a size of 3.5 μ (fig. 36).

*b. Prepupa.* During the fourth instar the Macrocentrus feeds on the outside of the host larva and then begins spinning its cocoon. In the spinning prepupa the fat-cells have attained almost their maximum size, ca. 50 μ (fig. 37).

About eighteen hours after the prepupa has begun to spin, communication is established between the lumen of the midgut with that of the hindgut, and the insect voids the excrement which has been accumulating in the midgut during larval life. From this time forward there are to be found in the central fat-cells, in addition to the acidophile spheres, spheres filled with basophile granules, and these spheres increase in size up to 10 μ (fig. 38) some of them are only granulated in part and the granulated portion partly or entirely encloses an acid-staining body entirely resembling the substance of the acidophile spheres (*b*, fig. 38). When stained with iron-haematoxylin, the granular spheres are a deep, opaque black and their granular structure is not so evident.

Those peripheral fat-cells which contained no acidophile inclusions during larval life may also at this time become filled with basophile granular spheres, but continue to remain free of the acidophile type (fig. 40).

In this instar, too, when spinning is completed and the large muscles of the thorax are just beginning to form, many of the thoracic fat-cells, especially the cells of the peripheral fat-body, undergo degeneration, in the case of the latter, without first developing any albuminoid spheres.

The degenerating cells appear as if undergoing phagocytosis, but it is difficult to determine whether such is really the case. It is true that the individual cells appear to break up into smaller spherical masses which are supposedly the results of phagocyte activity as described by Perez in the ant and in the wasp at a corresponding period of development. However, in Macrocentrus it is difficult to distinguish with certainty the leucocyte nucleus. The nuclei of the blood cells in this species are not large nor are they stained distinctively enough to be definitely recognizable if embedded in a mass of fat-cell material which contains chromatic bodies of diverse aspect, the results of chromatic degeneration.

At a somewhat later stage in the prepupa and in the pupa, the central fat-cells enclosed between the developing thoracic muscles are seen to degenerate in a way which entirely precludes the possibility of phagocytosis. The cell inclusions are gradually resorbed and the cells become smaller in size, and finally fall to pieces, the residue dissolving in the blood. Such cell fragments, usually still containing a few small inclusions, are found everywhere between the developing muscles in the pupa.

In sectioned material it is found that in the abdomen the fat-cells, together with the intercalated large urate cells, and peripherally the oenocytes, occupy the body cavity in the form of a solid mass (fig. 41), in which cell boundaries cannot always be distinguished except at the periphery of the mass where the cells may happen to be less densely aggregated. The fat-cells are at this time free from each other and have very delicate cell membranes, so that they are even more fragile than the cells of Pteronidea.

In making smear preparations it is found that the cells will remain entire if placed in a drop of normal salt solution on a cover-slip, but will rupture and disintegrate if the liquid on the slip is drained off with filter-paper or is allowed to dry with the intention of making a smear fixation possible. It is, therefore, certain that in the intact insect there is no extensive disruption of the fat-cells, especially since in later stages they are again visibly intact. Those fat-cells which survive pupation and are found in the imago are the same cells which were present in the mature larva and which have always maintained their identity.

*c. Pupa.* In the recent pupa (figs. 39, 40) we have similar conditions, illustrated by figures drawn in optical section from smears stained with haemalum. The nucleus (fig. 39) is distorted and has branches extending between the cell inclusions. Its border is not distinct, there is no definite membrane visible, and in the cytosome surrounding the nucleus are numerous small basophile granules and aggregations of such granules. Wherever cells have become ruptured in the process of making a smear preparation, the nucleus becomes partly freed from its hemmed-in position between the inclusions and is then seen to consist of a mass of chromatic granules, still retaining its deformed shape, but contained in a clear vesicle surrounded by a distinct membrane (fig. 42). This observation may indicate that the nuclear membrane does remain intact, although not discernible in sectioned material or in uninjured cells on smear mounts of this stage (figs. 38, 39).

However, the small basophile granules and aggregations of granules in the cytosome do resemble closely the granules in the nucleus. And when it is noted that here, as in Pteronidea, the advent of basophile granules in the cytosome is coincident with changes in the shape of the nucleus, we are not only again reminded that nuclear substance is taking an active part in the metabolic changes going on at this time, but the possibility suggests itself that formed particles may actually migrate out of the nucleus after disappearance of the membrane. Such an occurrence is by no means demonstrated by these observations, but still a theory of this kind appears to be not entirely gratuitous.

In the cytosome of the pupal fat-cells are again seen the two types of albuminoid spheres, the acidophile spheres (*a*, figs. 39, 41) and the basophile granular spheres (*b*, figs. 39, 41). The former are the same spheres which were formed during early larval life and which attained their maximum development in the prepupal instar. They are refracting, crystalloidal bodies which stain intensely with acid dyes. The basophile granular spheres are those which were formed during the prepupal stage, and which develop rapidly and become larger than the other type. Both kinds of spheres evidence signs of incipient dissolution. The acidophile spheres, especially in more advanced pupae, become corroded and excavated. In the granular spheres the basophile granules become more or less diffuse and indistinct, and minute clear vacuoles may appear within the sphere.

At this time there are also present, within the fat-cells, as well as between fat-cells, about the muscles and oenocytes, and in the blood, masses and droplets of a formless material staining with iron-haematoxylin (fig. 41, *c*) with the same intensity as the granules in the fat-cells. In the sections larger and smaller masses of this material are found and one encounters larger masses which have pseudopodia-like buds on the surface, indicating the breaking up of the masses into smaller globules which are found surrounding the mass and diminishing in size as they are farther removed, till in the free blood plasma they are reduced to the finest dust. The form of the larger masses indicates a liquid somewhat more viscous than the blood which has been coagulated in fixation, and it is probable that this material represents the reserves of the albuminoid spheres which have gone into solution and are secreted by the cells and after dissolving in the blood are used in nourishing the developing imaginal tissues.

*d. Late pupa and imago.* In the pupa and early imago the albuminoid inclusions gradually disappear from the cells, the large granular spheres dissolving most rapidly so that in imagos one day old only acidophile spheres remain in the fat-body. As the inclusions are used up, the fat-cells decrease in size and in imagos several days old the fat cells are small and contain no albuminoids at all, but only the fat-vacuoles.

At the time when the imago frees itself from the pupal skin a second defecation takes place which removes the residue remaining from the disintegration of the alimentary epithelium of the prepupal instar. This residue consists in part of a substance coagulable in fixatives and in part of a brownish oil which, although readily staining with sudan III, differs from the fat of the fat-cells in being insoluble in alcohol. It dissolves easily, however, in acetone. A few hours later the imaginal excrement consisting almost entirely of urate crystals begins to leave the anus, and is likewise deposited within the cocoon.

## THE FAT DURING THE METAMORPHOSIS

It has been reported by Perez ('12), Bishop ('22, '23), and others that, during the formation of the albuminoid granules, the fat-vacuoles almost entirely disappear, and it is thought probable that the fat is used up in the production of the granules.

In Pteronidea and Diphadnus it was found that fat-globules are present at all times, the quantity of fat in proportion to the other cell constituents, however, already suffers reduction in the last larval instar when rapid cell growth occurs and the fat does not increase in like proportion. During the prepupal stage there is some additional reduction of the fat-vacuoles which may indicate utilization of their contents in albuminoid production. In the fat-body of the imago, which contains almost nothing but fat, we probably have to do with the same fat which has survived until this time, while the granules have been used up, for now the fat-cells are much reduced in size and it is unnecessary to suppose the transformation of albuminoids into fat.

In Macrocentrus the fat present in the cells is always considerable in quantity, and it is difficult to determine whether any portion of it has been used in the production of albuminoids. In the peripheral fat-cells, in which large numbers of granular spheres are formed during transformation, there seems to be a reduction in the amount of fat (compare figs. 35 and 40).

The continued presence of large amounts of fat throughout metamorphosis in the central fat-cells of Macrocentrus, a form in which voluminous albuminoids are stored during larval life and in which relatively few and then only granular spheres are added during metamorphosis, compared with the apparently diminished amount observed in Pteronidea, in which many acidophile spheres are formed at this time may serve as additional evidence in favor of the view that fats are used up in the formation of albuminoids. It shows, too, that the fat is used mainly in the formation of those albuminoid spheres which are strongly acidophile and which also stain intensely with iron haematoxylin.

## THE URATES IN THE FAT BODY

Associated with the fat-cells and forming an important constituent of the fat-body are the excretory or urate cells.

### *1. Origin and development of the urate cells in Pteronidea*

In Pteronidea and in Diphadnus these cells are limited entirely to the central fat-body. They are present in the first larval instar, but do not attain their maximum development until the last larval instar and do not function at all until the insect has begun to spin. They are always found at the periphery of the fat-cells, in younger larvae entirely embedded, occupying a vacuole in contact with the cell membrane (figs. 7, 8) in older larvae, and in the pupa they occupy a depression in the cell (figs. 16, 18, 20, 25), so that their surface is exposed to the body fluid.

In the first instar, only a few of the fat-cells contain an embedded urate cell, at the end of the second instar the majority of fat-cells have at least one such cell and the numbers are found to increase as the larva becomes older, so that when about to transform nearly every fat-cell shows on its surface from three to six cells. In all save the last larval instar these cells are small, their nucleus is very condensed, somewhat smaller than that of the blood cells, and the cytoplasm is very scanty (figs. 7, 8, 13, 15).

During the last larval instar the nucleus enlarges and the cytoplasm increases in amount, and in the prepupa, as transformation begins, the cells begin to store concretions of urate and grow in size up to 35 μ (fig. 25). The urates in the functioning cells are present in the form of sphaerocrystals, as in most insects, and under the microscope appear dark by transmitted light and a brilliant white by reflected light.

As to the origin of the urate cells in the fat-body, there is no definite information in the literature. Some authors believe them to be transformed fat-cells which, after losing their fat-vacuoles, assume the urate-storing function, others that they are distinct cells which never metabolize fats. Berlese believed that the nuclei of the excretory cells are derived by division or budding from the fat-cell nuclei, and Anglas ('02) concurs in this view. As already indicated, the urate cells become associated with the fat-cells in increasing numbers throughout larval life, and the observations indicate that they are leucocytes which have become attached to, and finally embedded in, the fat-cells. Especially in the second instar are found here and there blood cells in contact with the surface of the fat-cells and at this time, too, the cells already embedded, or partly so, most closely resemble the leucocytes outside the cells. Once within the cell they show no further activity and never divide, but their cytoplasm becomes more reduced, and in the nucleus the chromatin condenses into a single chromatic mass. In this condition they remain until the last larval instar, when they grow larger in anticipation of their functioning during metamorphosis. As, during the prepupal stage, the sphaerocrystals of urate accumulate and grow, the cell increases in size, while the cytoplasm becomes very scanty and the nucleus enlarges.

At the time of the final molt and during the early imago stage, especially when the imago is still within the cocoon, the urates again disappear from the cells and are then found in the hindgut of the imago and are represented by the whitish material in the imaginal excrement.

### *2. Development of the urate cells in Macrocentrus*

In Macrocentrus, as already pointed out, excretory cells containing urate concretions are already present in the first larval instar. These cells are as large or larger than the fat-cells among which they are intercalated. They are found exclusively in the central fat-body, at first only in the abdomen, but later in the thorax also. During larval life these cells continue to enlarge and to amass urates (figs. 34, 36), and in the prepupa and pupa become exceedingly large and prominent, attaining a size of 60 to 70 μ, while the fat-cells are between 30 and 50 μ in diameter. The nucleus in these fully developed urate cells is greatly distorted, occupying the interstices between the urate granules in the central region of the cell.

Although two well-developed malpighian tubes are present in the larva and their lumen is filled with a secretion which seems to flow out between the large mass of glandular cells which forms a kind of plug filling the cavity of the hindgut and projecting slightly through the anus, the function of these could not be determined. The urates, as noted, remain in the excretory cells of the fat-body until the imago has emerged, and it is not until after the first day of imaginal life that they are removed from the fat-body and transported in solution to the alimentary canal through the malpighian tubes. During this time the imago still remains within the cocoon and the gradual disappearance of the concretions from the urate cells and their reappearance, again in the form of sphaerocrystals, in the hindgut and posterior end of the midgut is easily traced by fixing imagos from cocoons, at timed intervals, with absolute alcohol in which the urates are not soluble. While the transport of urates to the intestine is still going on, those already there continue to be voided through the anus in the form of a continuous strand of white material which accumulates in the cocoon.

### *3. Source and significance of the urate concretions*

The occurrence of uric acid or of its salts in the malpighian tubes and in the excreta of insects has been recognized since the discovery by Chaussier, in 1783, of an acid found in the excreta of the silkworm and the subsequent analysis of this substance by Brugnatelli, in 1815, according to Marchal's ('89) paper. Then, in 1817, Rengger found that this substance occurred also in the malpighian tubes and recognized in it an excretory product—an interpretation confirmed in the following year by chemical analyses carried out by Wurzer.

According to Leydig ('64), the occurrence of concrements of urate within the cells of animals was first described by Meckel in 1846, who found them in the kidney cells of Mollusca. In insects they were first reported by Faber in 1856, who found them in the fat-body of Sphecid larvae, and by Leydig, as observed in Lampyris and recorded in his Lehrbuch in 1857. Since that time urate concretions, occurring usually in the form of sphaerocrystals, have been described in many insects and their significance has been much discussed.

Berlese considered the urate concretions found in the fat-cells as products of intracellular activity, which cannot be eliminated during transformation because the malpighian tubes are not functioning at this time. Hollande ('14) believes that the urates are formed only as the result of metabolic processes going on within the fat-cells, that the urates represent by-products formed during the production of albuminoid granules by the breaking down of nucleoproteins into proteins and nucleic acid, which finally yields uric acid and is deposited in the form of urates. There are also other observations which support the view that urates are the result of the metabolic activity in the fat-cell.

In Calliphora (Dip.), according to Perez ('10), and in Galeruca (Col.), according to Poyarkoff ('10), as already pointed out, it is thought that the urates are stored within the albuminoid granules themselves, being visible as small embedded basophile particles, which stain readily with haemalum. In other Coleoptera and in Lepidoptera, Berlese and others have found urates within the fat-cells. Finally, in the Hymenoptera, the urate concretions are accumulated in separate cells which serve no other function, but which are closely associated with the fat-cells. Monodontomerus (Ichn.), as reported by Berlese, constitutes an exception; in this species the urates are found within the fat-cells.

Hollande ('14) believes also that urate accumulation in the fat-body does not depend upon the non-functioning of the malpighian tubes, because urates will pile up in the cells while the tubes are supposedly still functioning. Then it has been found by Philiptschenko ('07), according to Kreuscher, that the injection of urates apparently does not increase the amount present in the cells and that in certain insects, such as the Orthoptera (s. l.), which do not undergo transformation, we nevertheless have an accumulation of urates occurring.

Still it would be difficult to prove that all the urate is the result of transformations of material in the fat-cells, since in insects which do not produce albuminoids we yet find urates in the fat-body. It is obvious, therefore, that the metabolism going on in the remaining tissues, as well as that in the fat-cells, yields urate wastes.

It is also difficult to determine just when the malpighian tubes are functioning and when not. It seems better to consider the sudden beginning of urate deposition in the cells as a sign that the tubes have ceased to function, for in any case the great bulk of the concretions are formed at a time when the tubes are quite incapable of functioning. Then, too, when the tubes in the young imago of Macrocentrus begin to function, these accumulated urates, which make up at least one-third of the contents of the abdomen, are excreted by the malpighian tubes with surprising rapidity. So, in the Hymenoptera at least, the determining factor for urate deposition in the fat-body is the condition of activity of the malpighian tubes.

### 4. *Urates in non-holometabolic insects*

In the Thysanura and Collembola (Bruntz, '04, '08; Philiptschenko, '06, '07) and in the Orthoptera (Cuenot, '96), insects which do not undergo a complete transformation and in which the organs of elimination suffer no interruption of activity, it is difficult to understand why so much urate should accumulate in the fat-body. In Orthoptera (s. l.), according to Cuenot, very considerable quantities of urate are stored in special urate cells in the fat-body throughout the life of the insect and in old imagos may constitute a major portion of the fat-body. This is especially true of Blattids, Forficulids, Grillids, and Locustids. In these the urates are in the form of large sphaerocrystals similar in appearance to those in the Hymenoptera. In Mantids, Acridids, and in Gryllotalpa the urate cells ramify among the fat-cells, and the included crystals are comparatively small. Cuenot reports the entire absence of urates in the malpighian tubes except for small amounts together with uric acid which he finds in the white malpighian tubes of Gryllotalpa. Others have reported sodium urate in the tubes of Gryllus and Periplaneta, and calcium oxalate has also been reported in some forms.

The urates found in Orthoptera differ from those in the Pteronidea and Macrocentrus (Hymenoptera). When fixed with sublimate and sectioned and stained by ordinary methods, the orthopteran urate crystals are preserved on the slides, apparently being insoluble in water. They are dissolved, however, by Flemming's fluid, which contains chromic and acetic acids. In the Hymenoptera described in this paper the concrements are readily soluble in water and weak alcohols. May this difference in solubility be associated with the excretion or non-excretion of these compounds by way of the excretory organs? Perhaps not, for in Collembola, according to Philiptschenko ('06), are found water-soluble urates accumulating and remaining in the body. Might the fate of the urates be a mere matter of expediency? Their presence in the Orthoptera apparently does not inconvenience these insects, while in the more agile Hymenoptera they would surely be a handicap.

## THE ALBUMINOID INCLUSIONS

The accompanying table is an abridged compilation of observations recorded by various authors, on the time of appearance, development, and staining reaction of the albuminoid spheres found in the fat-cells. Since the data have been gathered from diverse sources, from observations made with different fixatives and stains, from studies in which the albuminoids have received unequal attention, it was found necessary, in order to make a comparison possible, to refer all the albuminoid inclusions to either of two groups.

The *basophile spheres* are those which assume a more or less intense violet color when stained with haemalum. In this group belong spheres which stain very strongly, approaching the color of nuclear substances, as well as those spheres in which the staining substance is more diffuse and is mingled with acidophile material.

The *acidophile spheres* include all those which are variously described as being either 'acidophile,' 'eosinophile,' 'cytoplasmic,' 'hyaline,' or 'crystalloidal.' They are again a somewhat heterogeneous group, since wide differences exist in their affinity for iron-haematoxylin and in their optical properties, but they have in common an entire lack of affinity for haemalum, at least never becoming violet when this dye is used.

### *1. The basophile substance*

An examination of the table will show that at least some albuminoid spheres which contain basophile material are present in the fat-cells of the great majority of the species which have been examined.

## TABLE 1

*Types of albuminoid spheres in the holometabolic insects*

| GENUS STUDIED (WITH SOURCE) | IN EARLY LARVA | IN MATURE LARVA AND PREPUPA |
|---|---|---|
| **Hymenoptera** | | |
| Pteronidea (this paper) | None | Basic (small granular), give rise to<br>Acid (with a basic exterior shell) |
| Macrocentrus (this paper) | Acid | Acid (the same as in larva)<br>Basic (coarsely granular) |
| Hylotoma (Berlese) | None | Acid (a few)<br>Basic |
| Calliroa (Berlese) | None | Basic |
| Monodontomerus (Berlese) | | Basic (feebly so)<br>Acid |
| Cynips (Berlese) | None | Basic<br>Acid (in peripheral parts of cells) |
| Nasonia (Tiegs) | Acid (small) | Acid |
| Pheidole (Berlese) | Acid (minute) | Basic (in central parts of cells)<br>Acid (in peripheral parts of cells) |
| Formica (Perez) | Acid | Acid |
| Polistes (Berlese) | None | Acid (small), becoming<br>Basic (nearer the nucleus) |
| Apis (Berlese) | Minute | Basic (feebly so), becoming<br>Hollowed (feebly basic) |
| Apis (Bishop) | None | Basic, becoming<br>Hollowed (slightly basic) |
| **Lepidoptera** | | |
| Pieris (Berlese) | Basic (small) | Basic<br>Acid (confined to central fat-cells) |
| Pieris (Nakahara) | Acid | Acid, becoming<br>Basic |
| Hyponomeuta (Berlese) | None | Basic, in some cells, near nucleus<br>Acid, in peripheral parts of cells |
| Hyponomeuta (Hufnagel) | None | Acid (crystalloidal)<br>Basic (slightly) |
| Bombyx (Berlese) | None | Acid |
| Vanessa (Hollande) | None | Basic, becoming<br>Acid |
| **Coleoptera** | | |
| Sitodrepa (Berlese) | Acid (small) | Acid<br>Basic (slightly) |
| Aphodius, Coccinella, Lampyris (Berlese) | Acid (small) | Acid |
| Galeruca (Poyarkoff) | Acid | Acid, some becoming<br>Hollowed (with basic exterior) |
| Dytiscus (Kreuscher) | None | Acid, and also some<br>Basic (according to l. c. fig. 13) |
| **Diptera** | | |
| Calliphora (Berlese) | None | Acid, becoming<br>Basic (some in form of 'pseudonuclei') |
| Calliphora (Perez) | None | Acid, some of which become<br>Basic (slightly); others, 'pseudonuclei' |

This basophile substance, because of its staining affinities, its usual propinquity to the nucleus, and the nuclear changes observed in some forms at the time of its appearance, is thought to be derived from the nucleus. Concerning its origin and significance, a number of theories have been put forward. In general, either the basophile material is considered as the starting-point, the spheres when first formed being basophile and later developing into spheres which are less basic or even acidophile, or the acidophile type may be considered as the original kind of albuminoid sphere which later may acquire a more or less basophile character.

In the honey bee, Bishop ('22, '23) describes solution and rupture of the nuclear membrane followed by an invasion of the cytoplasm by basophile nuclear granules, induced in part by the pressure of the fat-vacuoles which have migrated inward from the cell periphery. These granules grow by the addition of materials absorbed from the cytoplasm, becoming less and less basophile, until finally they stain only with cytoplasmic dyes. It appears, according to this author, that the basophile material contains an active agent or enzyme which acts upon substances in the cytoplasm, converting them into the albuminoid spheres. Each basophile nuclear granule gives rise to one albuminoid sphere, the basophile material diffusing and becoming less stainable as the sphere grows in size.

Hollande ('14), in a careful study on the formation of albuminoids in Vanessa (Lepid.), also describes the appearance of basophile granules in the region about the nucleus. These he designates as 'chromatinoid substances'; they grow and transform into 'protein' spheres staining with lichtgrün. The protein spheres finally transform into 'crystalloid albuminoids.'

Berlese ('99–'01, '02), especially in his description of the fat-cells of Calliphora (Dip.), describes the intracellular digestion of albuminoid substances. These substances, derived from the histolysis of larval tissues, are taken into the fat-cells where they form acidophile spheres, which, as they approach the nucleus, are acted upon by enzymes derived from the nucleus, and the basophile granules in the spheres are the visible form of these enzymes. These albuminoid spheres containing basophile granules were named 'pseudonuclei' by Berlese, since they were mistaken by certain previous workers for the nuclei of leucocytes which had penetrated the fat-cells or for the nuclei of daughter cells formed within the fat-cell.

More recent careful investigations on Calliphora by Perez ('10) largely confirm the observations of Berlese. However, the interpretations are different, Perez, as already pointed out, maintaining that the basophile particles referred to are urate granules.

The observations reported in the present paper indicate that, in Pteronidea and in Diphadnus, the progenitors of the mature albuminoid spheres are small basophile spheres which grow by the accumulation within their interior of acidophile material. As this growth proceeds, the granular basophile material remains as a peripheral layer which becomes less and less stainable. The striking changes in the form of the nucleus, its reduction in volume, and the condition of the nuclear membrane, all indicate that the bulk of the material of the nucleus is being transferred into the cytoplasm. Since this happens during or just previous to the first appearance of basophile spheres in the cytoplasm, it seems reasonable to consider this material from the nucleus as being the substance out of which the spheres are formed.

The observations on the fat-cells of Macrocentrus indicate a relation of nuclear changes to the appearance of basophile spheres similar to that found in the two Tenthredinids. In their subsequent development, however, the basophile spheres of the Ichneumonid behave differently. They not only remain more strongly basophile, but the densely staining granules in them are rather coarse and are evenly distributed in a more scanty matrix which never becomes strongly acidophile.

From the information so far obtained, the basophile substances found in the cytosome of the fat-cells during metamorphosis must be considered as having their origin in the nucleus. They have passed out of the nucleus by diffusion through the nuclear membrane, or in some forms apparently as discrete particles following a temporary dissolution of the membrane. Functionally, these basophile substances may possibly represent a kind of enzyme necessary either to initiate the formation of albuminoid spheres or, when such spheres are already present, to catalyze intracellular digestion.

### 2. *The acidophile substance*

As already indicated, acidophile spheres may develop out of smaller, basophile spheres. This is seen especially well in Pteronidea. In the cells of this species the developing spheres become internally acidophile, while the basophile material remains only as a thin superficial layer which in material fixed in warm B15 is entirely invisible. In this same material it is also observed that the acidophile substance first passes through a stage in which it is unstainable with iron-haematoxylin—a condition which parallels the descriptions of Hollande, already referred to. Finally, however, these acidophile spheres all stain intensely with iron-haematoxylin and, when fully developed, especially in the abdomen, may become refracting bodies which stain translucently black and resemble the crystalloids described in Macrocentrus.

Hufnagel ('18), in her description of the fat-cells in Hyponomeuta (Lepid.), also finds crystalloidal acidophile albuminoids which are especially large and numerous in the abdomen. In the thorax they are more varied in size and less crystalloidal in appearance.

In Macrocentrus, acidophile albuminoids are found very early in larval life. Such an early appearance of these bodies is uncommon in insects, but has been recorded, notably by Perez ('02), in the ant. These acidophile spheres in Macrocentrus are rather strongly refracting, stain translucently with iron-haematoxylin, and appear to be crystalloidal in structure. There is no basophile material associated with their origin, nor do they appear to bear any direct relation to the nucleus. During transformation a few of these spheres may become partly embedded in or enclosed by the developing basophile spheres formed at this time.

Our information regarding the acidophile inclusions in the fat cells of insects leads to the conclusion that they are passive reserve substances, formed gradually during larval life through the activity of the surrounding protoplasm, or formed very rapidly at the time of metamorphosis in association with a basophile substance of nuclear origin. In either case, in their final form they may become more or less refracting.

### 3. *The fate of the albuminoids*

Whatever their mode of origin or their final appearance, the albuminoid inclusions all share the same fate. They are dissolved within the cells and the resulting products diffuse into the blood during prepupal, pupal, and early imaginal life. In Pteronidea the more highly refracting spheres remain in the cells longest, the others seem to be more readily subject to dissolution. In Macrocentrus this relation is again observed, the crystalloids dissolve more slowly, becoming corroded on the exterior, and are still present in young imagos in which basophile granular spheres are no longer found. The latter also dissolve in a different manner, they become lighter, more diffusely staining, and contain small vacuoles within. Degenerative processes seem to be going on throughout the mass of the sphere as if due to internal causes, or, if the dissolution is due to agents in the cytoplasm, that these are able to diffuse into the basophile spheres, while they attack the crystalloids only on the surface.

### 4. *Other theories concerning cell inclusions*

*a. The granula theory.* According to the theories of Altmann and his followers, certain granula play an important rôle in the transformation of substances within cells. Metabolic exchanges involving fats, proteins, and carbohydrates are all believed dependent upon the activity of these granules.

We have found that in insects definite granular material derived from the nucleus is associated in an active way with the formation of albuminoid spheres during metamorphosis. And if fat is also used in the building up of these spheres, then the somewhat basophile granules described are certainly of importance not only in transformations involving proteins or albuminoid substances, but also in the metabolism of fat. The albuminoid spheres themselves, however, cannot be regarded as analogous to the granula of Altmann.

Bogdanoff ('98) describes granules occurring in certain cells in the bone marrow of frogs. These granules transform into fat, fusing together into larger masses, and the cells thus become fat-cells. Since these granules stain intensely with iron-haematoxylin and hold this stain as tenaciously as do the nucleoli of the cells and also yolk granules from hen's eggs, mixed with albumin and sectioned, Bogdanoff concludes that they represent nuclear secretion products resembling yolk. Pospeloff ('98) believes that the albuminoid granules described in Dytiscus are associated with fat formation. And Popoff ('10), in imagos of the housefly, believes that the chromidia, whose origin is traced to the nucleus, give rise to the fat by a series of regressive changes.

Since, however, in the fat-cells of insects no phenomenon is more evident than the deposition of fat-droplets and the disappearance of the same at times when no granules at all are visible in the cells, it is evident that fat exchanges are not dependent upon the presence of granules and that universality cannot be claimed for the theories of Altmann and others regarding such an association.

*b. The origin of yolk.* The appearance of basophile material in the cytoplasm of the fat-cells of insects at the time of formation of cell inclusions calls to mind the theories of R. Hertwig ('07) and of Goldschmidt ('04) regarding the origin and function of chromidia and also the numerous papers which have appeared describing the formation of the yolk spheres in the developing eggs of various animals, especially coelenterates and echinoderms. According to Hertwig and Goldschmidt, the chromidia or the chromidial apparatus has its origin in the nucleus, it is the 'trophochromatin' which has passed into the cytoplasm to take part in the purely vegetative functions of the cell. Such an extrusion of chromatic particles from the nucleus has since been described as occurring in egg cells by a number of authors. Smallwood ('09), Schaxel ('10, '11), G. T. Hargitt ('13, '16), and Danchakoff ('16) find, in addition, that the yolk spheres in the egg develop out of these chromidia, or at least in close association with them. Ludford ('21), however, finds the yolk is formed by Golgi bodies, and not by the fragments of nuclear material which are also present in the cytoplasm.

Other authors, including King ('08), Jörgensen ('10), and Beckwith ('14) (on amphibia and coelenterates), also describe the formation of yolk spheres, but find that there is no active participation of chromatic nuclear particles involved. They believe, as did Carnoy and Lebrun ('98), that the nucleus may take part in yolk formation by liberating dissolved substances which may react with the cytoplasm.

*c. Mitochondria.* Finally, the formation of albuminoid spheres has been linked with the activity of mitochondria.

Vejdovsky ('25), in studying the fat-cells of the honey bee, has apparently confused some of the cell inclusions. The empty vacuoles in sectioned material which in the fresh cells contain globules of fat he refers to as 'hyaline vacuoles.' The spheres which stain uniformly with iron-haematoxylin and red with fuchsin he considers as fat and describes their mode of origin. These granules, he states, are formed in relation to minute mitochondria which are associated with little uncolored globules. The uncolored portion transforms into fat which forms as a little calotte upon the plastomerite or mitochondrial body. The illustrations showing groups of these rounded objects together giving rise to large spheres of fat or fatty substance are suggestive of the granular spheres and their association with achromatic material as seen in Pteronidea and in Macrocentrus, the fatty calotte being the unstained matrix; the plastomerites, the granules. The 'fat' with which

Vejdovsky's observations deal stains light red with fuchsin and gray with iron-haematoxylin, it is not osmiophile and resists the solvent action of the reagents used in preparation—reactions which are all characteristic of the elements described by others as albuminoid granules and whose nature is indicated by their affinity for eosin, picric acid, and other dyes and the yellowish color they yield in the xanthoproteic reaction. Vejdovsky believes his observations to correspond to those of Fauré-Fremiet, who observed osmiophile granules in Paramecium. In these granules, blackened with osmic acid, when treated with alcohol or other fat solvents, a part, supposedly representing the fatty portion, fades out, while the remainder, representing the mitochondrial moiety, remains. Since in both Pteronidea and Macrocentrus it was observed that, in osmium preparations of fat-cells, the blackened fat-droplets may either persist or disappear when mounted in balsam, sometimes entirely, sometimes in part, depending upon the degree of osmification, it seems that this method is not always entirely dependable.

It might be noted here that in the present investigation on Pteronidea it was found that, in unstained sections mounted in balsam, the albuminoids in cells fixed with Champy or Flemming were not blackened by the osmic acid, although the large fat-droplets in the same cells were entirely black.

Paillot and Noel ('26) have also described the formation of albuminoids as associated with mitochondria. In Bombyx and in Pieris these authors describe mitochondria in the form of short threads, upon which appear swellings which gradually enlarge to form the albuminoid spheres. When these spheres become resorbed the mitochondria are again visible. .

Similarly, in other forms and in other types of cells, mitochondria have been thought responsible for the formation of cell inclusions, such as yolk and fat (Arnold, '14; Schreiner, '15; Saguchi, '20; Hibbard, '22). The mitochondria involved are described in some forms as originating in the nucleus—a circumstance which, according to one theory (Duesberg, '12, cited from Schreiner, '15), would indicate that they are not true mitochondria. None of the substances described in the present paper can be interpreted as mitochondria, since they persist in cells which have been fixed with solutions containing considerable acetic acid, such as Bouin's. However, it is interesting to note the way in which mitochondria (in this case derived from fat-droplets) give rise to yolk plates in the Echinoderm eggs described by Hibbard ('22). In these eggs yolk is observed to accumulate within the interior of the mitochondrial granules, and as development continues yolk plates are formed upon the surface of which the mitochondrial substance is still seen. Such a development parallels somewhat the method of formation of the albuminoid spheres in Pteronidea and Diphadnus, although the substances involved differ.

## INTERPRETATIONS OF INTRACELLULAR CHANGES

### *1. Fat and glycogen*

Information regarding the significance of these intracellular changes has as yet not attained beyond the recognition of the more obvious inferences which may be derived from the observations recorded. It is found that the fat-cells during larval life develop as do the other tissues of the body and, in addition, are specialized as reservoirs of reserve food materials. The principal inert vehicle for stored energy is the fat. Glycogen may be present, as pointed out by Nakahara ('17), diffusely, but was not found to exist, in the shape of formed bodies definitely recognizable by the iodine or Best's carmine tests, in Pteronidea.

### *2. The albuminoids*

An additional reserve element is the albuminoid granules which, as already pointed out, do not display any regularity in the time of their appearance in the different orders. As may be seen from Berlese's papers, from Henneguy's ('04) account, and from individual observations since, albuminoids are never present in young dipterous larvae, but make their appearance at the beginning of transformation. In Lepidoptera they are found in the larva of Pieris, but not in the larva of Bombyx and Hyponomeuta, which has suggested correlation with the amount of silk produced. In Coleoptera certain phytophagus and a caprophagus species have albuminoids in the larva, while in the carnivorous Dytiscus they do not form until the end of larval life. In Hymenoptera they are found in younger larva of Tropinoma, Formica (Formicidae), Cynips (Cynipidae), Nasonia (Chalcidae), and Macrocentrus (Ichneumonidae). In other forms, Calliroa, Pteronidea, Diphadnus (Tenthredinidae), Polistes (Vespidae), and Apis (Apidae), they appear first at the beginning of the metamorphosis. In the carnivorous Neuroptera granules are formed during larval life, while in the Phryganeids (Trichoptera) they do not appear until transformation. Finally, in the termites, according to Feytaud ('12), granules have been found in young individuals, but not in old, mature ones. From these data it is not possible to associate the appearance of albuminoids with larval feeding habits.

### 3. *The significance of the albuminoids*

The functional significance of albuminoid production during metamorphosis has generally been thought to lie in the conversion of all fat-cell reserves into one type of reserve element which is subsequently resorbed and passed in soluble form into the blood to nourish the developing tissues.

In Pteronidea and in Macrocentrus it is found that the formation of albuminoids associated with metamorphosis does not begin until absorption of food has ceased. In Macrocentrus digestion still continues in the spinning prepupa and the new albuminoids do not appear till the end of the spinning phase. In Pteronidea the prepupal alimentary canal is empty even before spinning begins and again it is noted that nuclear changes and granule formation begin as soon as the alimentary canal has been emptied. Such conditions indicate that fat-cell changes are not initiated until these cells have ceased assimilating materials absorbed by the intestine—

until the cells have attained their maximum growth. In the prepupa of Pteronidea, which is beginning to spin, granule production in the fat-cells is well under way (figs. 22 to 24). There is at this time very little evidence of histolysis of larval organs, the only signs of such changes are seen in the anterior part of the midgut epithelium where cells are beginning to loosen and in the cells of the segmental ventral abdominal glands which consist of invaginated portions of the hypodermis functioning only in the larva.

This evidence is ample to demonstrate that the albuminoids are not formed out of materials derived from the histolysis of larval tissues, as has been suggested, for the histolysis which has occurred up to the time when the granules are over one-half formed is too slight to be of significance. In a prepupa in which the albuminoids have fully developed (fig. 25) we find that histolysis is well under way, though not nearly half the larval tissues have been removed. In the head are seen the vacuolated remains of the larval mandibular muscles surrounded and mingled with proliferating muscle nuclei and in the abdomen new muscles have begun to form, although the larval muscles have just begun to show signs of disintegration. The acini of the spinning glands are still present, but are beginning to degenerate, the imaginal salivary glands are forming about the degenerating larval ones, and in the hypodermis there are everywhere many mitotic figures indicating a rapid proliferation of imaginal cells. At this time the fat-cell reserves are not yet extensively called upon, for the histolysis of the larval tissues must yield considerable food material.

At the end of the prepupal instar and in the pupa and imago, however, when histolysis has been completed, food for growth and to satisfy energy requirements must be obtained from the fat-cells which are now filled with albuminoids and fat. Since in insects as well as in other organisms the fat-cells may act as organs of reserve, taking up fat and again liberating it into the blood when required without the production of albuminoid granules, the meaning of such granules at the time of metamorphosis remains obscure. The suggestion has been made (Popoff, '08) that the formation of cell inclusions, such as yolk, fat, and glycogen, is a sign of depressed ('gelähmte') cell activity, that it is an expression of the inability of the cell to complete the organic synthesis of absorbed materials. In regard to the formation, in egg cells, of deutoplasmic inclusions derived from the protoplasm, he states (l. c., p. 373) that "verschiedene organische Zellbestandteile verfallen allmählich in folge der schweren Funktionsstörungen einer regressiven Metamorphose und geben nach dieser Weise den Ursprung niederer Stufen organischer Verbindungen: es bilden sich Dotter, Fett, u. s. w." In hymenopterous fat-cells we might also refer to the changes going on during the metamorphosis as being regressive in nature in that the albuminoid spheres produced are chemically more simple in composition than the protoplasm out of which they are in part formed. There is, however, nothing to indicate a depression in cell activity. On the contrary, the large scale transformations of fat and cytoplasmic materials which take place and the nuclear changes accompanying these indicate a tremendous acceleration in cell activity. This phenomenon of the transformation of cell constituents suggests that the fat-cells are not only reservoirs of reserve food, but that they also actively make over the reserves which they contain into the albuminoid spheres as representing a form which may possibly be more soluble, easily transported, or more readily available for assimilation—a form which is at all events the one actually utilized for tissue growth during the metamorphosis of the insect.

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## SUMMARY

1. A comparative study of published statements concerning the microscopic anatomy and the postembryonic development of the fat-body in the various holometabolic orders, combined with the observations recorded in the present paper, indicate that the fat-body in the Hymenoptera is characterized by, 1) the absence of mitotic divisions in fat-cells during postembryonic life, except in the recently hatched bee larva; 2) the presence in the fat-cells of fat and albuminoids, the latter in some forms appearing early in larval life, in others not until the inception of the metamorphosis; 3) the disappearance during the metamorphosis of many fat-cells, by histolysis after the resorption of their inclusions, and in some forms by the possible phagocytosis of many cells; 4) the survival of larval cells to form the imaginal fat-body; 5) the existence in all forms recorded, except Monodontomerus (Ichneumonid.) of separate excretory or urate cells.

2. The excretory function is taken care of during the transformation in Pteronidea and Diphadnus and, during the entire preimaginal life in Macrocentrus, by special urate cells closely associated with the fat-cells. The accumulation of the water-soluble concretions of urate in these cells is coincident with the period of non-functioning, as urate eliminators, of the malpighian tubes. After the final ecdysis these urates are eliminated rapidly by means of the imaginal malpighian tubes.

3. The urate cells in the fat-body of Pteronidea have their origin in leucocytes which have become embedded in the surface of the fat-cells during the course of larval life.

4. In the albuminoid inclusions found in the fat-cells two types of substance are distinguished: *a*) Basophile material, of nuclear origin, found in the cytosome in the form of small granules or granular aggregations and appearing only during the metamorphosis. In Macrocentrus this material takes part

in the formation of large albuminoid spheres consisting of masses of, at most, feebly acidophile material filled with numerous coarse basophile granules. Such spheres never become strongly acidophile. In Pteronidea the basophile substance, found in the cytosome in the form of small granular basophile spheres, gives rise to large, strongly acidophile spheres. In these spheres the original basophile material may remain as a thin, lightly staining layer on the surface. Before the acidophile spheres are fully developed, they pass through certain intermediate conditions, as indicated by their changing affinities for iron-haematoxylin. *b*) Acidophile material in the form of albuminoid spheres which may, as in Macrocentrus, originate during larval life in the absence of any basophile substance, or which may be formed, as is the case in Pteronidea and Diphadnus, at the time of the metamorphosis and in intimate association with a basophile substance of nuclear origin.

It is thus found that, when albuminoids are formed slowly during larval life, they may exist from the beginning in the form of crystalloidal, highly acidophile spheres. But when formed rapidly, as occurs during the metamorphosis in Pteronidea, they are formed in association with basophile material of nuclear origin and undergo visible transmutations, as recognized by their staining affinities, before finally becoming highly acidophile.

5. The fat accumulated during larval life in Pteronidea and Diphadnus appears to suffer a diminution in quantity during the metamorphosis, at a time when the acidophile albuminoids are being formed in the cells. In Macrocentrus, in which copious acidophile albuminoids in addition to the fat-globules are formed during the course of larval life and in which there are no additional acidophile albuminoids produced during the metamorphosis, there is no apparent diminution in the fat-content of the cells. This observation substantiates the theory that fat may be used in the production of albuminoids. It also demonstrates particularly that it is the acidophile inclusions, rather than those which remain predominently basophile, in whose formation fat is utilized.

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## EXPLANATION OF PLATES

The figures on plates 1 to 4 are reproductions, reduced one-half, of drawings made with the aid of a camera lucida. A Spencer microscope with a 1.8-mm. achromatic objective was used, and all drawings were made at table level under the following conditions: for plate 1, ocular 12x, tube length 165 mm.; for plates 2 and 3 (except figs. 29 to 31), ocular 6x, tube length 147 mm., and for plate 4, ocular 10x, tube length 173 mm.

Plates 5 and 6 contain reproductions of photographs taken with the aid of a Leitz apparatus, using a Zeiss microscope with a 4-mm. adjustable apochromatic objective and a 6 compensating ocular. The magnification of the individual exposures is approximately that of the corresponding drawings made from the same objects, as reproduced on plates 2 to 4.

Unless otherwise noted, the figures are from serial sections, cut in paraffin at 6 to 8 $\mu$.

### ABBREVIATIONS

*a*, acidophile albuminoid sphere
*b*, basophile albuminoid sphere
*c*, constituent of fat-cells and of blood, probably the product of dissolution of albuminoids
*h*, hypodermis
*l*, leucocyte
*ur*, urate cell

NOTE. The clear areas in the drawings of fat-cells represent fat-vacuoles from which the fat has been dissolved out by the reagents used in preparing the slides.

## PLATE 1

### EXPLANATION OF FIGURES

Cells from the fat-body of larvae of Pteronidea ribesi (Scopoli) fixed in warm Allen's B15, stained with iron-haematoxylin. × 1000.

1 Peripheral fat-cell of embryo, two leucocytes are also seen.

2 Central fat-cells of the same embryo.

3 Peripheral fat-cell of a first instar larvae, several leucocytes are attached to the fat-cell.

4 Central fat-cells from the same larva.

5 Peripheral fat-cells from the same larva.

6 Peripheral fat-cells from another larva in the first instar.

7 and 10 Central and peripheral fat-cells, respectively, of a larva in the second instar; central fat-cell contains an embedded urate cell.

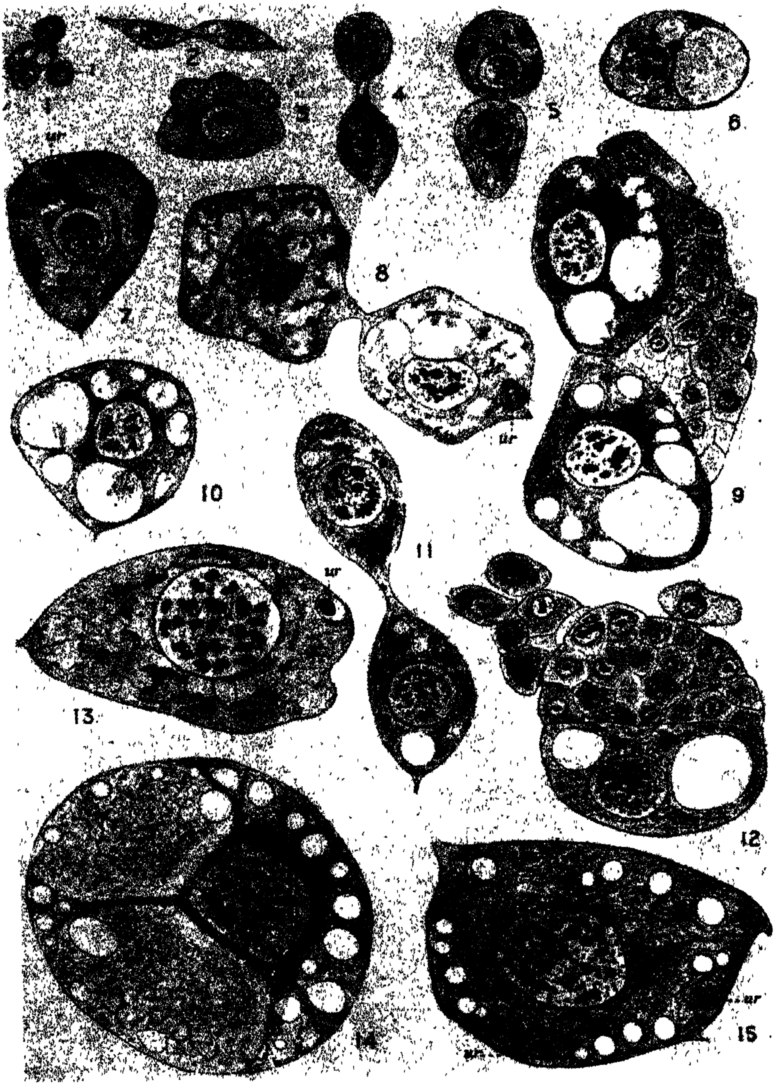
8 and 9 Central and peripheral fat-cells of another larva in the second instar; a colony of leucocytes is attached to the peripheral fat-cells.

11 Cells from the central fat-body of a third-instar larva.

12 Peripheral fat-cell with a colony of leucocytes attached to it, from the same larva. Some of the leucocytes are developing into free amoebocytes.

13 and 14 Cells from central and peripheral fat-body, respectively, of a fourth-instar larva.

15 Central fat-cell of another larva in the fourth instar.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## PLATE 2

### EXPLANATION OF FIGURES

Cells of the fat-body from larval and prepupal instars of Pteronidea ribesi (Scopoli) (figs. 16 to 19, 21, 22) and from prepupal instar of Diphadnus appendiculata (Hartig) (fig. 20). Stained with iron-haematoxylin, except figure 21 which is stained with haemalum and orange G. × 500.

16 and 17 Cells from central and peripheral fat-body, respectively, of immature last larval instar. Fixed in Flemming's fluid, fat-droplets blackened by osmic acid. Photograph of the cell in figure 16, in figure 44.

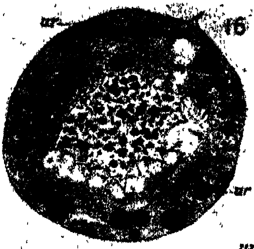
18 Half of a central fat-cell from a full-grown last larval instar, fixed in warm Allen's B15. Photograph of entire cell in figure 46.

19 Half of a central fat-cell from prepupal instar which had just molted and not yet begun spinning. Fixed in Champy's fluid.

20 Central fat-cell from the prepupal instar which was beginning to spin its cocoon. Fixed in warm Allen's B15.

21 Cell of approximately the same stage as figure 20. Fixed in absolute alcohol.

22 Cell of central fat-body from thorax of a spinning prepupal instar. Fixed in warm Allen's B15. Photograph in figure 45.



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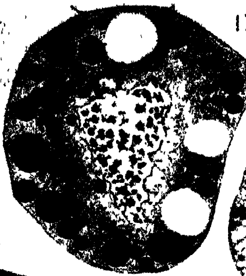

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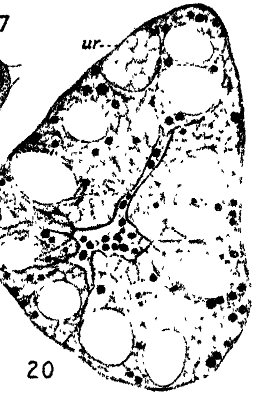

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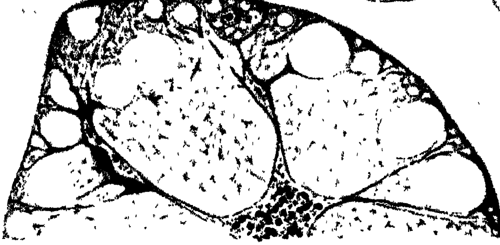

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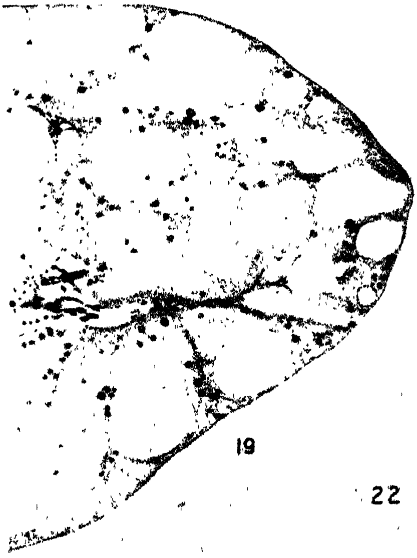

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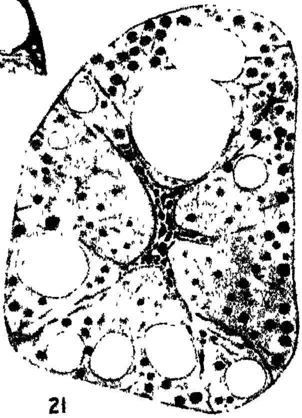

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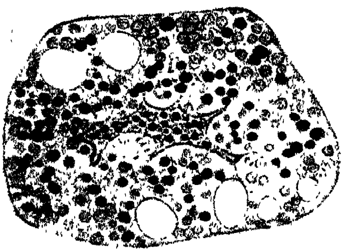

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## PLATE 3

### EXPLANATION OF FIGURES

Cells from the fat-body of prepupal, pupal, and imaginal instars of Pteronidea ribesi (Scopoli) (figs. 23, 24, 26, 27, 29, 31) and of Diphadnus appendiculatus (Hartig) (figs. 25, 28). Figures 23 to 28, × 500; figures 29 to 31, × 120.

23 and 24 Cells from the peripheral fat-body of thorax and abdomen, respectively, of a spinning prepupa. Fixed in Allen's B15, stained with iron-haematoxylin.

25 Half of a central fat-cell from abdomen of a prepupa three days old. Fixation and staining as above.

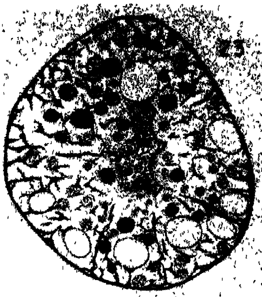
26 Half of a central fat cell of a two-day-old prepupa. Fixed in absolute alcohol, stained with haemalum and orange G.

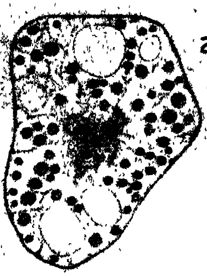
27 Half of a central fat-cell from the abdomen of a prepupa. Fixed in Champy's fluid, stained with iron-haematoxylin. Photograph in figure 47.

28 Fat-cell from the thorax of a young imago still within its cocoon. Fixed in Allen's B15, stained with iron-haematoxylin. Photograph in figure 48.

29 A lobe of the fat-body from an imago still in its cocoon. Drawn from fresh material stained with sudan III.

30 and 31 Fat-cells from the abdomen of a somewhat older imago. Smear preparation; fixed in formalin, stained with haemalum and sudan III.





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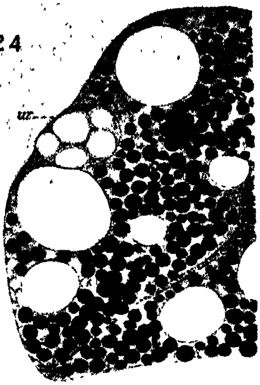

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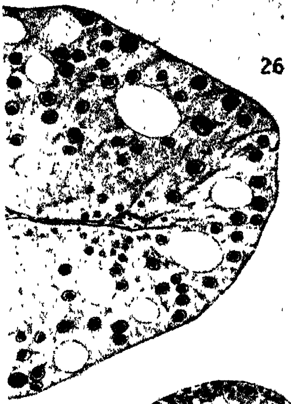

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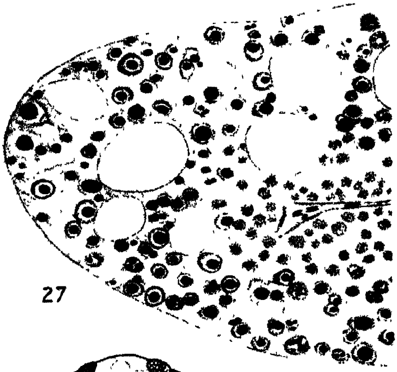

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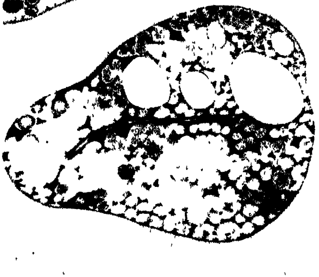

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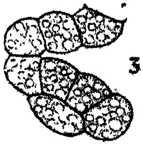

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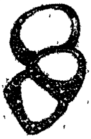

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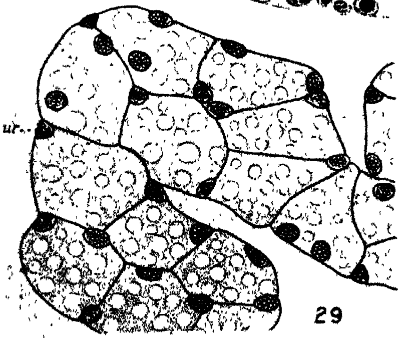

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## PLATE 4

### EXPLANATION OF FIGURES

Cells from the fat-body of Macrocentrus ancylivora Rohwer. Figures 32 to 36, 41, are from sectioned material fixed in warm Allen's B15 (except fig. 32, fixed in Schaudinn's) and stained with iron-haematoxylin. Figures 37 to 40, 42, are from smear preparations fixed in 4 per cent formalin, and stained with haemalum and orange G. × 930.

32 Cells from central fat-body of first-instar larva.

33 and 34 Cells from peripheral and central fat body, respectively, of second instar larva. In figure 34, the middle cell in the upper row is a urate cell.

35 and 36 Cells from peripheral and central fat-body, respectively, of a third-instar larva. The cell on the left is a urate cell. Photograph in figure 43.

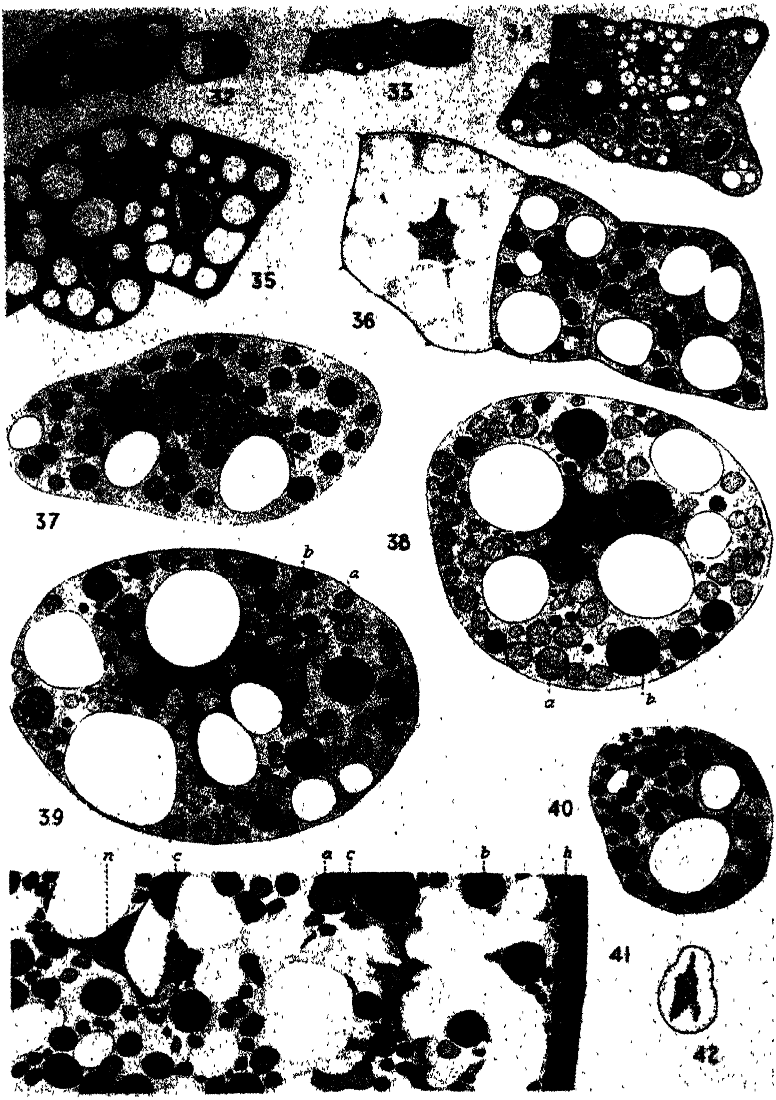
37 Central fat-cell of prepupal instar; spinning under way.

38 Cell from central fat-body of a prepupal instar at a later stage. Photograph in figure 49.

39 and 40 Cells from central and peripheral fat-body, respectively, of a white pupa.

41 Small portion of central fat-body of a white pupa. Photograph in figure 50.

42 Nucleus from central fat-cell which had been injured in preparation of smear.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## PLATE 5

### EXPLANATION OF FIGURES

Microphotographs of fat-cells of Pteronidea ribesi (Scopoli) and Macrocentrus ancylivora Rohwer; fixed in Allen's B15 (except figure 44, which was fixed in Flemming's fluid) and stained in iron-haematoxylin.

43 Central fat-body of Macrocentrus larva in the third instar. Includes the cells drawn in figure 36.

44 Central fat-cell of immature last larval instar of Pteronidea. The same cell as in figure 16.

45 Central fat-cell of a spinning prepupa of Pteronidea. The same cell as in figure 22.

46 Central fat-cell from a full-grown Pteronidea larva of the last larval instar. The same cell as in figure 18.

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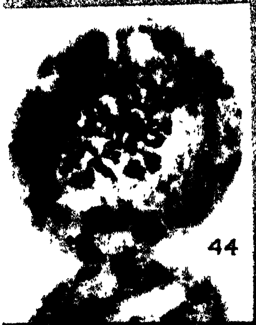

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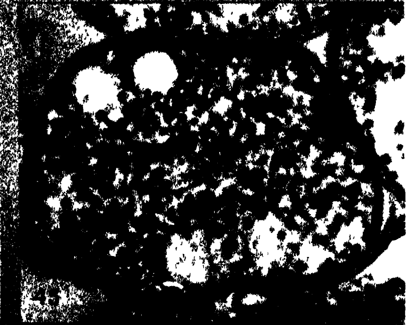
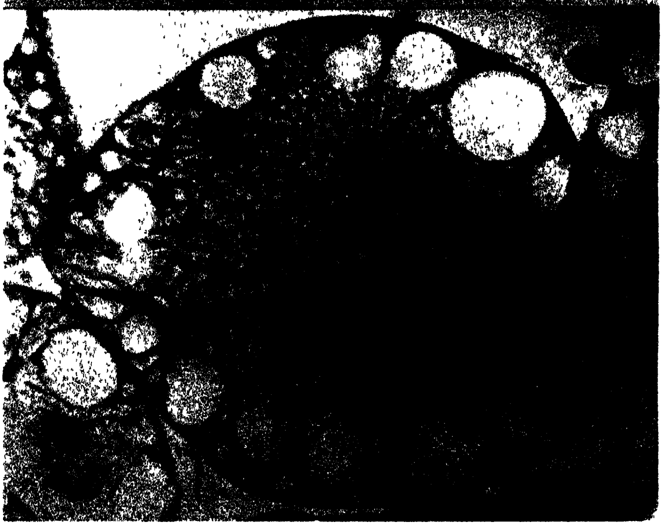




# PLATE 6

## EXPLANATION OF FIGURES

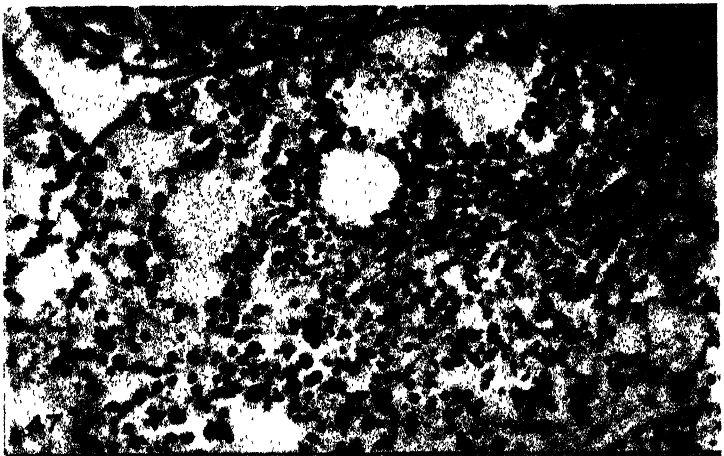
Microphotographs of fat-cells of Pteronidea ribesi (Scopoli) and Macrocentrus ancylivora Rohwer. Figure 49 is from a smear fixed in formalin and stained with haemalum. The others are from sectioned material stained with iron-haematoxylin.

47 Central fat cell from the abdomen of prepupa of Pteronidea; fixed in Champy's fluid. The same cell as in figure 27.

48 Fat-cell from the thorax of a young imago of Pteronidea still within its cocoon; fixed in Allen's B15. The same cell as in figure 28.

49 Central fat-cell from prepupa of Macrocentrus. The same cell as in figure 38.

50 A portion of the central fat-body of a white pupa of Macrocentrus. The same as in figure 41.



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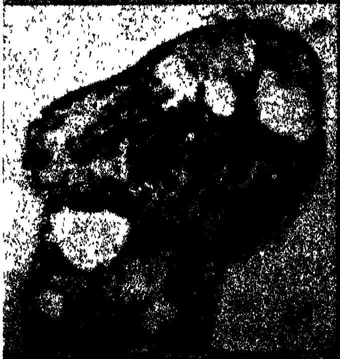

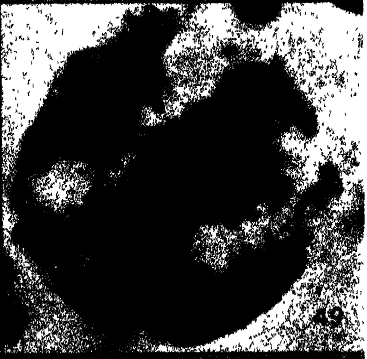

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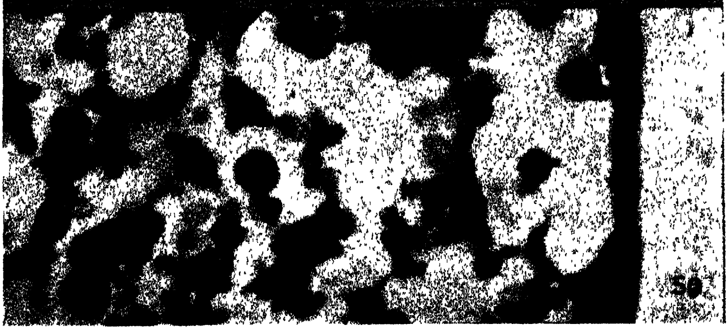

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# A SEXUALLY MATURE TURBELLARIAN RESEMBLING MÜLLER'S LARVA

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SIXTEEN FIGURES

AUTHOR'S ABSTRACT

This paper deals with the description of an organism, externally resembling Muller's larva among the polyclads, collected in the plankton of Monterey Bay, California, during the spring and summer months. Internally, the organization of the various systems is unique. In some respects it bears a certain resemblance to a polyclad in early stages of development, in other regards it approaches the rhabdoceles, more particularly the Acoela. Its more exact relationships are obscured by the fact that, although the larger specimens are not more than 0.7 mm. in length, the sex organs, both male and female, are fully developed and functional. Furthermore, the plan of these systems is unlike that of any turbellarian hitherto described. Whether this organism represents a case of paedogenesis or is a fully developed adult is unknown at present, but in any event the various systems are described and an attempt is made to give it a fairly definite systematic position.

The term 'persistent larval types' was applied by Huxley to those organisms which in their adult condition approach, either in their external form or internal organization or both, the young stages of living representatives of various phyla. Such undoubted examples are comparatively rare, unfortunately, but where, like Trochosphaera among the rotifers and Appendicularia among the chordates, they do occur they constitute an important bit of evidence relative to the evolutionary history of the class or phylum to which each belongs. In the present paper we have to do possibly with another member of this class of organisms which may serve as a clue to the origin of the rhabdoceles. On the other hand, it is equally possible that it may be a paedogenetic Müller's larva. In any event, it differs in several important respects from any other known form, combining features which are reminiscent of the rhabdoceles in some respects and of the polyclads in others.

In connection with a general biological survey, a series of plankton hauls was made in Monterey Bay during the summer of 1926, and various invertebrate larvae were mounted for demonstration purposes. Among these were a number of Müller's larvae, representing several different polyclads, all of which were sexually immature, with the exception of one species, and closely corresponded in their organization to the well-known figures of Lang. These exceptional individuals, upward of fifty in number, were in various stages of development from those without apparent gonads to others of larger size, eleven in number, with functional germ cells and accessory reproductive organs. The entire collection was killed in hot Bouin's solution, and of the larger specimens six were stained in Delafield's haematoxylin and sectioned, while the remainder was mounted entire between coverglasses which permitted the examination of both sides of the body.

The average length of six mature individuals in a living state and in a relaxed condition was 0.628 mm., the smallest being 0.57 mm. and the largest 0.729 mm. The shape of the body, as may be seen in the figures, is an exact duplicate of Müller's larva among the polyclads, there being eight ciliated lappets with the same characteristic arrangement, including the ciliated bands between the median dorsal lappet and the dorsolateral pair. Furthermore, the mouth occupies the same position between the ventrolateral processes. In fact, it was only after considerable experience that I was able to distinguish the present species from definite polyclad larvae, occasionally captured in the plankton, of the same size, shape, and much the same type of coloration.

The epithelial or hypodermal layer as usual comprises a single layer of cells belonging to several categories. No especial study has been made of these in detail, nor is the material killed in Bouin's fluid particularly favorable. Nevertheless, it is readily possible to determine that over the body generally the most abundantly represented type of epithelial cell is relatively slender, usually four or five times higher than wide, and bears on its outer surface a coat of cilia. Its boundaries, even in the most favorable material, are indistinct, and the nucleus, usually located in the basal half of the cell, is relatively compact. In these respects it stands in marked contrast to the ciliated lappet cells and the ciliated band uniting them where the cell elements have a somewhat greater diameter, relatively clear boundaries, a lightly staining centrally located nucleus, finely granular cytoplasm, and a coat of cilia three times the length of that covering the body generally. Furthermore, the cells of the lappets appear to possess an unusually definite arrangement; at least those of the median frontal process form not less than six definite transverse rows, judging from the position of their nuclei as seen in whole mounts.

In fresh 'squeeze preparations' epithelial gland cells are visible in the epithelial layer, both on the dorsal and ventral surfaces, their glass-like secretion appearing with great distinctness in some specimens. This appears to be the same type of cell which in sections is seen to contain a basally situated nucleus, and, more distally, a darkly staining material often in the act of escaping at the surface. It may be added that the individuals of this species often cling with great tenacity to the substratum.

Rhabdite cells are abundantly distributed over the body generally, and, as usual, they possess basally situated nuclei, and on their free surfaces bear a coat of cilia. The contained rhabdites are smooth, spindle-shaped glass-like bodies whose greatest length is, approximately, 0.008 mm. In common with the polyclads, the rhabdite cells are elements of the epithelial layer, and never have been found to lie beneath it in the parenchyma.

Over eighty years ago, Quatrefages ('45) discovered in the planarians what have been termed tactile organs in the form of immobile protoplasmic processes at least twice the length of the cilia covering the body generally, and, according to Keferstein ('68), these are fairly evenly distributed over the body. In the present species they were visible in living specimens, but disappeared in preserved material. Hence it is not possible at the present time, with stained specimens, to determine their number or their exact distribution. In one living specimen, represented in figure 1, eight of these hairs were visible in the anterior two-thirds of the body, the one in the midline being paired. In another the only variation concerned the two hairs in the midline which were distinctly separated. In the posterior third of the body the number varied from five to seven, the additional members being added to the region bordering upon the median line. In another individual at least three additional organs of this type were seen on the dorsal surface of the body posterior to the level of the mouth, while two others were situated close to the midline in the brain region. It is probable that others exist, but the opacity of the body and the movements of the living animal have prevented more exact observations. It may be added that the average length of each hair is 0.014 mm. Two living individuals were treated with methylene blue, but no light was thrown on the innervation of these organs.

The basement membrane in this species is, neglecting minor details, essentially the same as that of the polyclads as described by Lang ('84). In two specimens particularly this structure is unusually clear, and consists first of highly branched cells, apparently anastomosing and certainly giving the impression of a distinct network which underlies the outer epithelial covering over the entire body, including the lappets. Nuclei in the species studied by Lang were deeply staining; in the present case they possess but little chromatin, and it is only with difficulty that they can be detected. Furthermore, the network on the ventral side of the body is of a more delicate character with more open meshes than on the dorsal side. In common with the polyclads, these branched cells are embedded in a lightly staining matrix, homogeneous in character and extremely tenuous over the body generally, but becoming distinctly thicker in the interior of the lappets. In these last-named organs especially the muscle fibers frequently are distinct, and at several points their extremities are anchored in the intercellular material. Many of the finer details are obscure, but as far as they have been determined they support Lang's statement that the basement membrane "eine Art Hautskelet, das dem Körper zur Stütze und speciell den Muskeln zur Anheftung dient."

No especial study has been made of the body musculature, but where it is clearly defined in sections its general plan conforms to that of polyclad larvae. Longitudinal muscle fibers, beneath the epithelium and spaced at intervals greater than their diameters, course from one end of the body to the other. Diagonal muscles in the same general situation are somewhat less numerous. Dorsoventral strands also are distributed throughout the body, especially in the region of the pharynx—a fact observed by Lang in the young of Yungia aurantiaca ('84, pl. 37, fig. 1).

A detailed study was made of the parenchyma in the hope that distinguishing features might be discovered which would serve to differentiate it from the endodermal layer. As Lang states for certain species of polyclads, the parenchyma cells appear to form a continuous reticulum, and in the present species, owing to the absence of a limiting membrane about the endoderm, it seemingly is impossible to determine its exact limits. Accordingly, no definite statement can be made at this time regarding the presence or absence of food or vacuoles in its substance.

The color of the body varies from dull green to slaty gray. Squeeze preparations demonstrate that the pigment involved is located in the parenchyma, never in the overlying epithelium. In the fresh state the granules can be seen to extend through the mesenchyme in the form of slender threads, occasionally with branching processes as though incorporated in definite cells. In preserved material all traces of coloring matter disappear, and its relation to the parenchyma remains uncertain.

The mouth, as previously mentioned, is situated between the median ventral lappets and communicates with a pharynx of the simplex type. The component cells of this last-named organ present much the same appearance as those of the lappets, although their average height is distinctly less. No special gland cells are visible at any point. In individuals with little or no food in the digestive tract the lumen of the pharynx communicates with a relatively large space, possessing a comparatively sharp internal border. Even in well-fed specimens the boundaries of the gut cavity at several points are fairly distinct, while in other situations a few slender outgrowths or branches, especially in the anterior half of the body, can be traced from the main cavity into the adjacent intestinal tissue. In one individual the intestinal cavity was more slender than the one represented in figure 12, and the cilia of the pharynx passed without a break into others which covered at least three-fourths of the inner surface of its digestive tract. In the remaining specimens the cilia were wholly restricted to the pharynx.

In several individuals the cavity of the digestive tract contained food, consisting of diatoms, an unknown species of flagellate, and several other unicellular organisms of minute size and unknown relationships. In some instances these lie freely in the lumen of the gut, whose walls in the vicinity are clearly defined. In other cases the nutritive substances are enveloped in pseudopodial processes, and in what is apparently an intracellular position other food materials are buried at varying distances from the digestive cavity. Thus digestion appears to be wholly intracellular. Finally, as in many rhabdoceles, no line of demarcation is distinguishable between the intestinal tissue and the parenchyma.

In rare instances delicate fibers have been found attached to the pharyngeal wall, and may be looked upon as possible dilator muscle cells. At other points there are somewhat unsatisfactory indications that the pharynx is also provided with a scanty coat of circular fibers. There is also another structure, conspicuous even in the smallest specimens, which in all probability is the homologue of the proboscis fundament of polyclad larvae. It presents the form of a clearly defined group of cells, more or less circular in cross-section (figs. 12, 14), completely encircling the oesophagus (fig. 8, *pr*). In a few specimens it appears to be partially surrounded throughout the greater part of its course by a clearly defined sheath. On its outer surface a few longitudinal muscle cells are visible, while the cells more centrally located are so closely aggregated that their true character cannot be determined. In all of these respects there is a close agreement between this organ and the rudimentary proboscis, as described by Lang for stage C or D of Müller's larva. Anteriorly, in the region of the penis, it is attached more or less superficially to muscle cells which have been traced anteriorly a short distance into the gut tissue. A few other fibers attach to its surface on the right side and extend far out toward the margin of the body.

No trace of an excretory system has been discovered either in living material or in sections.

The brain is a comparatively large body, nearly spherical in form (figs. 8, 12), located approximately halfway between the mouth and the forward end of the body. In one of the largest specimens, from dorsal view, it measured 0.063 mm. by 0.082 mm. On its anterior and lateral surfaces several groups of ganglion cells are plainly visible, but the destinations of the nerves leading outwardly from these are tantalizingly difficult to trace. The median ganglion, whose cells in the most favorable material usually stain more faintly than the usual nerve elements, sends a number of nerves forward to the end of the body. The ganglionic group more laterally placed continues as a thin cord as far forward as the anterior eyes where it becomes lost to view. A lateral branch in one specimen appears to unite with the longitudinal lateral nerve cord shown in figure 9. This also appears to be the case with the nerves leaving the other two pairs of ganglia attached to the brain.

A pair of stout ganglionated cords arises from the posterolateral surface of the brain, and in whole mounts can be traced to a point about opposite the mouth. Beyond this limit they are indistinguishable, but in sections they have been seen to unite in the midline posterior to the female genital pore. A strong commissure connects these at the level of rior to the mouth, its anterior extensions reaching a point about midway between the pharynx and brain on each side of the midline. Hence, whether of single or double origin, the functional vas deferens exists in the form of a U.

On the left side of the body opposite the mouth the vas deferens develops a short branch which pursues a short course anteriorly and enters the seminal vesicle, whose position and relation to other organs are shown in figures 5 and 8. In four specimens it exists as an almost spherical organ packed with sperms. A short canal leads from its anterior face into the base of the penis, and a sheath of delicate muscle fibers coats its external surface. At its point of entry into the ejaculatory duct it unites with the canal from the accessory or granular gland (Körnerdrüse), a globular organ consisting of a few cells with faintly staining nuclei and an equally faint secretion. The remaining features of the male reproductive system are represented in figure 5, where the penis is shown as a conical structure tipped with a delicate cuticular stylet, and surrounded by a sheath which opens close to the midline beneath the median ventral lappet.

Several peculiar features exist in connection with the female reproductive system. In the first place, three pairs of ovaries are fairly evenly spaced along the sides of the body. On the average, each measures 0.03 mm. in diameter in the sexually mature animal, each is surrounded by a definite sheath, and at a point usually opposite to its attachment to the oviduct each contains a small mass of cells whose divisions result in the formation of the oocytes. In a few cases there are signs of degeneration among these last-named elements, and the soluble products may serve as a food supply, but otherwise there appears to be no indication of special nutritive or yolk-bearing accessory cells.

Each ovary, as may be seen in figure 13, is attached to a slender oviduct, which extends beyond the rearmost ovary, and in the midline becomes continuous with its counterpart of the opposite side (fig. 8). Lateral to the pharynx a branch of this tube is developed on each side of the body, and likewise becomes united to its fellow in the midline slightly posterior to the female genital opening. From this last-named point of union a single canal extends anteriorly, and, after developing an antrum femininum, opens to the exterior on the ventral surface of the body posterior to the external mouth opening. A small group of cells, corresponding possibly to the shell gland of other species, is attached to the distal extremity of the unpaired oviduct. No trace of a special uterus has been discovered nor of a seminal receptacle.

Regarding the relationships of the present species, or Graffizoon, the name of the genus created for its reception, there are two obvious possibilities. It may be, in its fully developed condition, an adult descended from ancient ancestors resembling the modern Müller's larva, or it may represent a case of paedogenesis. Personally, I am inclined to accept the latter view, but even though this does prove to be the correct interpretation of the facts, there yet remains the problem of its more exact position in the scheme of classification. According to Lang ('84), and the later revision of the polyclads by Bock ('13), no species of this order has been described, so far as I can discover, in which the genital pores are separated by the mouth opening. Furthermore, the question of its cotylean or acotylean alliance must remain unanswered for the present, although Bock states that "die Müllersche Larvenform ist nur bei den Cotylen nachgewiesen." Also, whether the gut becomes clearly differentiated in later stages, if such there be, and what other transformations may ensue are also unknown.

A comparison of a typical Müller's larva (fig. A) with Graffizoon demonstrates, in the first place, that externally their resemblance is strikingly close. In the form of body, position, and structure of the ciliated lappets, the location of the mouth, and the number of eyes, distributed into two main groups, they are practically identical. With regard to their internal organization, on the other hand, Graffizoon presents a complex of unique characters along with others that are recognizable in polyclads or their larvae. For example, the epithelial elements, the basement membrane, and the general body musculature are typical of both, while the digestive system appears to be more nearly allied with that of the Acoela. There is a possibility, however, that, as regards this last-named system, the two in reality may be more nearly akin than at first appears.

Both Lang ('84) and Surface ('07) have shown that in the polyclads the functional endoderm cells in very early stages become amoeboid and absorb yolk fragments, after which the endoderm constitutes a single layer. In his figures of stage D, corresponding most closely to Graffizoon, Lang represents a spacious gut cavity, without definite pouches, bounded by a single epithelial layer, richly ciliated and extending from the brain to the posterior end of the body. In later stages cells appear to migrate from the gut and, although details are lacking, they form branches which develop into the characteristic polyclad intestine. This stage with few gut branches may represent the condition existing in the larger specimens of Graffizoon. Attention was called earlier in the account to the fact that in one individual especially four or five pairs of relatively indefinite extensions of the gut cavity extended laterally and became lost to view shortly before they reached

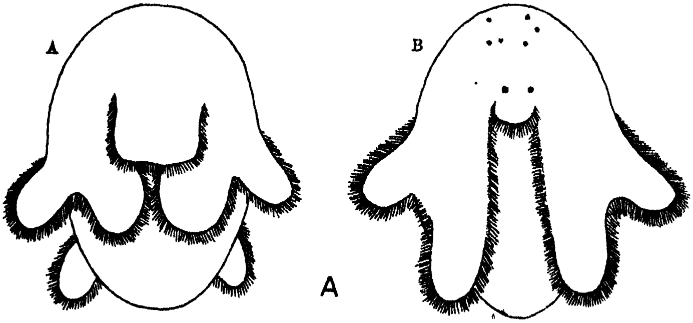

Fig. A Ventral (A) and dorsal (B) views of Muller's larva in the development of Yungia aurantiaca. After Lang.

the margins of the body. Also the ciliated coat of the main cavity penetrated into these, but could not be followed with certainty throughout their extent. In two other individuals the nuclei of the gut wall were less dense than those of the adjacent parenchyma, and other nuclei of the same type were visible here and there far out toward the surface of the body. Since this last-named feature was not an invariable occurrence, it may be of no especial significance in this connection, but it is suggestive if the following hypothesis proves to be correct.

According to Lang's observations, the cells of the gut may lose, during certain stages of their activity, all traces of cell membranes, and fuse into a syncytial sheet. The ciliated coat likewise becomes transformed into pseudopodial processes. In Graffizoon these same phenomena certainly occur, and, in addition, the protoplasm adjacent to the gut cavity is vacuolated, the vacuoles becoming larger as the surface of the body is approached. There is, however, an important difference. In the polyclads a basement membrane clearly defines the outer borders of the endoderm; in Graffizoon no trace of it has been discovered. If, however, the endoderm cells of a polyclad with stubby outgrowths from the main gut were to fuse, develop pseudopodia and vacuoles, and lose their basement membrane, the resulting appearance would duplicate, I believe, that existing in Graffizoon.

Whether such a theory will account for the state of affairs in the Acoela is problematical. Surface ('07), commenting upon the fact that in Planocera inquilina all of the digestive tract "arises from a portion of the posterior cell of the fourth quartette," makes the following comment:

> This peculiar development of the alimentary canal in the polyclads offers the suggestion that it may be a step toward the development of such forms as the acoelous rhabdoceles, in which the alimentary canal is altogether absent. But the embryology of these as well as other turbellaria present such great variations from the type found in polyclads that it is useless to speculate along this line.

This may indeed be the true explanation, but, on the other hand, it is equally possible that the larval endoderm may persist in the adult, and, becoming amoeboid and syncytial, develop a state of affairs resembling that in Graffizoon. But, as Surface remarks, speculations without embryological data are futile.

In this connection the pharynx plicatus rudiment (figs. 8, 12, 14) is of interest. Lang has traced its development from the stage in which it exists in the form of a solid ring of mesoblast cells surrounding the oesophagus to the functional organ. At one stage, D, its appearance and position agree almost exactly with this organ in Graffizoon, as may be seen if Lang's figure (pl. 38, fig. 4) of this stage is compared with figure 14 in the present paper. At this time we have little or no information regarding the phylogenetic origin of this type of proboscis. It may have arisen from a simple sphincter muscle, and in this condition it may exist in Graffizoon. Or, if we are dealing with a case of paedogenesis, it is equally possible that the proboscis rudiment is in an undeveloped condition. Whatever may be the exact explanation, it is certain that the digestive tract is functional, but whether the pharyngeal ring is active or passive in the feeding process remains uncertain.

Returning to the resemblance which Graffizoon bears to the rhabdoceles, it may be urged that if it were devoid of lappets its polyclad character, or, more definitely, its close relationship to Müller's larva doubtless would remain unsuspected. In this connection a suggestion of Lang is decidedly germane. In discussing the relationships of the Acoela—the Rhabdocoela or at least the Alliocoela being considered to be derivatives of the triclads and these of the polyclads—he raises the following question:

> Ist es denn nicht auch möglich, dass die Acoelen geschlechtsreif gewordene Jugendstadien alter Stammformen der Turbellarien sind? Wir wissen ja, dass nicht selten, z. B. gerade bei Ctenophoren, schon Larven geschlechtsreif werden. Bei dieser Auffassung wäre die Thatsache ebenfalls verständlich, dass die Acoelen in manchen Organisationsverhältnissen mit Bezug auf die übrigen Turbellarien ein einfaches, ursprüngliches Verhalten darbieten.

According to this view, the Acoela possibly may be paedogenetic turbellaria, but at the same time it must be admitted that such a theory rests upon hypothetical evidence to a large degree.

There is also another possibility, equally supposititious. From Lang's studies especially it has become apparent that a fairly close resemblance exists between Müller's larva, the ctenophores, and the trochophore. Furthermore, we can scarcely escape from the belief that this deep-seated similarity is to be explained on the theory that all of these organisms are the descendants of an ancient pelagic ancestor; and, what is more important in this connection, this ancestral type was probably sexually mature. May we not assume, therefore, that the rhabdoceles, or at least the acoelous component, are the direct descendants of this pelagic ancestral type rather than that they have been derived from some larval stage of this old stem form. According to this view, the rhabdoceles are rather to be looked upon as complete adults rather than paedogenetic organisms.

The reproductive system of Graffizoon is perhaps in closer agreement with that of the polyclads than with other turbellaria. In both the testes are ventral, the ovaries dorsal, and, as usual, there is successive hermaphroditism, as Lang terms it, the spermatozoa ripening first. The male reproductive system might belong either to a rhabdocele or polyclad, while the female system seemingly holds an intermediate position. No rhabdocele, so far as I can learn, possesses more than two ovaries, and as soon as these organs can be recognized in the developing polyclad—with a body length ranging from 5 mm. to 9 mm. in Monterey Bay species—they number at least fifty pairs. This, however, is not a fundamental difference. With a body length of approximately 0.6 mm., it is not surprising that the female germ cells in Graffizoon are relatively few. Furthermore, the union of the ovaries by means of an oviduct dorsal to the one leading to the exterior has its counterpart in Oligocladus sanguinolentus as figured by Lang ('84, pl. 23, fig. 3). In this species the ovaries are relatively numerous and the oviducts form an anastomosing system, but, what is important in the present case, they communicate across the midline.

As previously noted, the characteristics of Graffizoon can be explained on the theory that it is an adult organism directly descended from ancient pelagic ancestors. No proof to the contrary exists. A few medium-sized specimens were kept in aquaria for nearly a month, but no suitable food supply could be discovered, and further development failed to take place. The chief objection to this line of argument, however, is that the resemblance to Müller's larva is so close that it appears to be more reasonable to look upon it as a case of paedogenesis. Until this matter is settled, it is certain that the exact status of this species cannot be determined. Attention has been called to the fact that Müller's larva is characteristic of only the cotylean polyclad so far as known, and it therefore becomes more probable that Graffizoon belongs to this tribe; but I am unable to find a description of any genus in which the mouth separates the genital pores—a character which in all probability is unchanging in later stages if there be such. In view of these facts, the following classification is proposed.

### *Graffizoon,*[1] *new genus*

With external form of body as in Müller's larva. Mouth about middle of ventral surface. Pharynx of the simplex type, leading into a comparatively large main gut, apparently provided with a few lateral branches, whose walls lack a basement membrane and consequently are indistinguishable from the parenchyma. Female reproductive system comprises three pairs of ovaries with genital duct opening posterior to mouth. Testes, numbering about eighteen pairs, connected with U-shaped vas deferens, terminating in distinct seminal vesicle. Penis, provided with granular gland, slender, tipped with chitinous stylet, with sheath opening anterior to mouth. Eyes, two anterior and six in brain region. Brain relatively large, distinct, with at least one pair of ventral longitudinal nerves and a dorsal plexus.

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[1] Named in honor of my friend, the late Dr. L. von Graff.

Type of genus, Graffizoon lobatum.

The diagnosis of this genus is such that it cannot be included in any existing family. Accordingly, a new one, Graffizoidae, is herewith proposed with the characters of the genus and Graffizoon as the genotype. Provisionally, at least, it may be included in the polyclad tribe, Cotylea.

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## PLATE 1

### EXPLANATION OF FIGURES

1 Graffizoon lobatum, dorsal view; actual length, 0.63 mm.

2 Same as above, ventral view; arc-like band represents ciliated groove.

3 Young specimen; length, 0.2 mm.

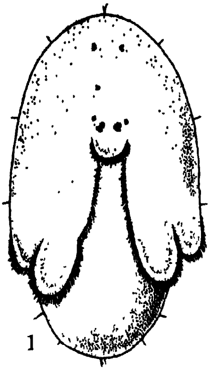
4 Older specimen, lateral view; length, 0.31 mm.

5 Median sagittal section through penis, prostate gland (*pg*) and seminal vesicle (*v*).

6 Median sagittal section through antrum femininum and shell gland (*sg*). *p*, possible proboscis rudiment; *v*, vas deferens; ciliated tract represents posterior border of pharynx.

7 Section through immature testis and vas efferens.

8 Slightly diagrammatic plan of the male and female reproductive systems. *b*, brain; *m*, mouth; *ov*, ovary; *pr*, supposed proboscis rudiment.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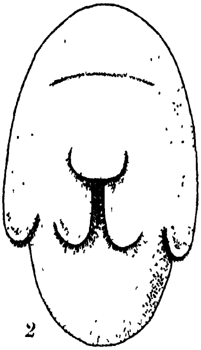
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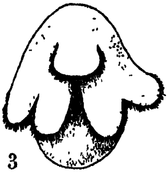
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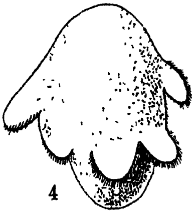
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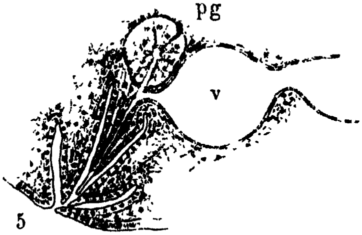

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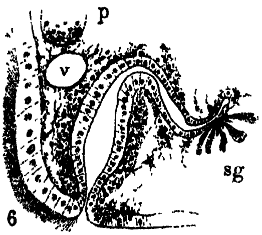

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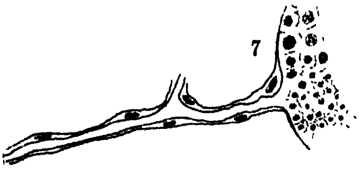
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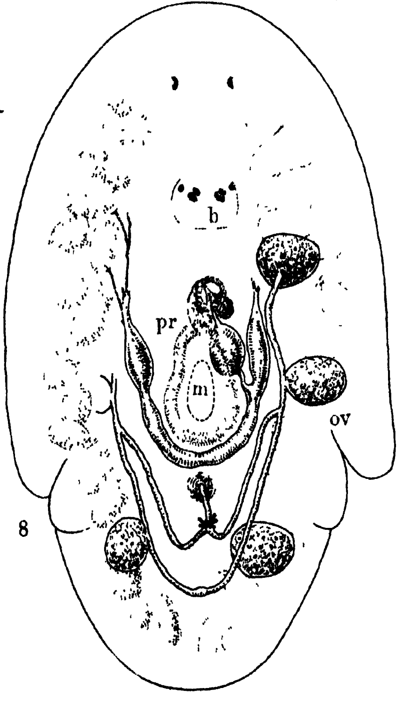

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## PLATE 2

### EXPLANATION OF FIGURES

9 Ventral view of nervous system; broken lines represent probable course of nerves.

10 Longitudinal section of gut from region of pharynx to point near posterior end of body; food material in lumen and buried in surrounding tissues.

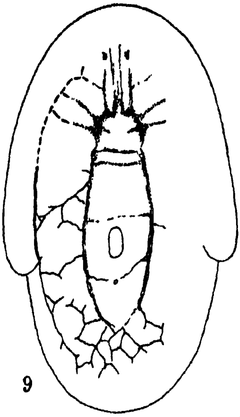
11 Rhabdites; actual length of largest, 0.008 mm.

12 Median sagittal section through entire animal; brain, two eyes, and portion of male reproductive system anterior to mouth; antrum femininum and one testis located in posterior half of body.

13 Section through an ovary and portion of oviduct; size of ovary, 0.06 mm. by 0.08 mm.

14 Transverse section through body at level of mouth; supposed rudiment of proboscis immediately dorsal to vas deferens (*v*); two ventral testes and one dorsal ovary represented; beneath innermost testis is ventral nerve cord, while another is attached to inner ends of cells of ciliated tract extending from median dorsal lappet to dorsolateral lappets.

15 Section transverse to ventral ciliated groove; nuclei beneath epithelial cells belong in large measure to ganglion cells; *e*, one of the anterior eyes.



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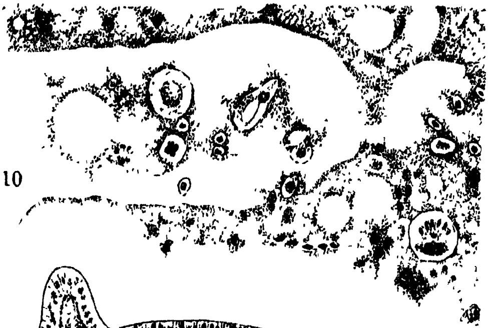

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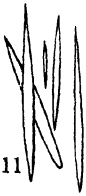

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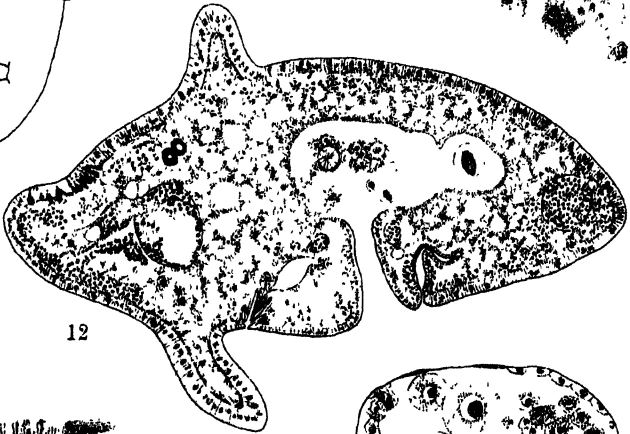

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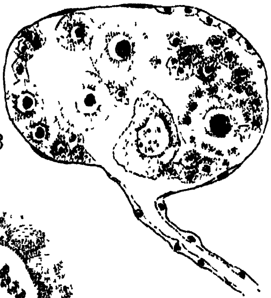

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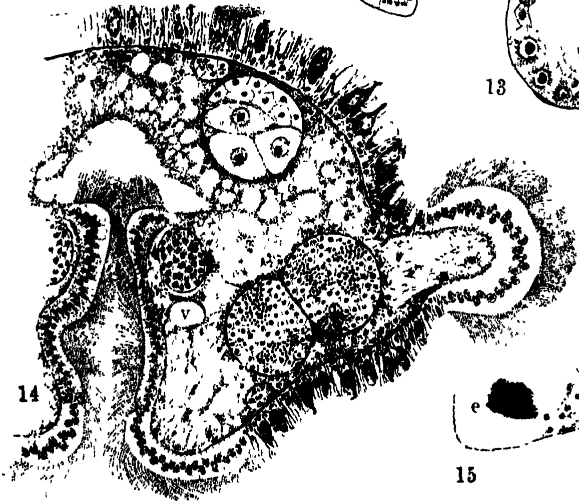

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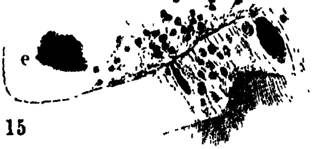

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# THE CRANIAL MUSCULATURE OF THE TOADFISH (OPSANUS TAU)

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ELEVEN FIGURES

AUTHOR'S ABSTRACT

The highly specialized cranial musculature of the toadfish is characterized by the following features

1. Absence of intermandibularis and branchiomandibularis muscles
2. Presence of levator premaxillaris muscle
3. Very large branchial chamber, the outer wall of which is formed by seven branchiostegal rays connected by a strong fascia provided with muscles (oblique levators and adductors)
4 Highly developed masticator muscles (adductor mandibularis and pterygoids)
5 The rectus abdominis, sternohyoid, and hyohyoid muscles are attached by a median aponeurosis to the hyoid and basibranchial elements and directly to the hypobranchial cartilages, this muscle complex depresses the buccal floor in opposition to the geniohyoid
6 The pelvic fins are in the jugular position.
7 Two narrow muscles connect the cleithrum with the fourth ceratobranchial
8 The cranial musculature is obviously adapted to a carnivorous habit and particularly for increasing respiratory capacity under asphyxial conditions

## INTRODUCTION

Although the toadfish (Opsanus tau) is well known to biologists, only a few papers are to be found that deal with its morphology. Miss Clapp ('98) described the "lateral-line system of Batrachus tau" and Gudger ('08) wrote, "The habits and life history of the toadfish." A few embryological papers have been written on this aberrant teleost, Ryder ('82), Clapp ('91), Gill ('07), and Tracy ('26).

The author wishes to express his appreciation for the kind and helpful suggestions continually given by Dr. Henry C. Tracy and Dr. Homer B. Latimer.

The present paper aims to supply a brief account of the cranial muscles of Opsanus tau, which may be available for comparative purposes or for future developmental studies.

Since the terminology of muscles in fishes has not been thoroughly standardized, the author deemed it desirable to construct a chart somewhat similar to Vetter's ('78). In this chart are tabulated in a comparative manner, for convenient reference, the cranial muscles of various fishes that have been described. The earliest work used in this paper is that on Perca fluviatilis by Owen ('66). Other well-known contributions to the cranial anatomy of fishes were made by Fürbringer ('75), Vetter ('78), and McMurrich ('84). Very extensive work has been done by Allis ('97 and '03) on such familiar forms as Amia calva and Scomber scomber. The most recent work is that of Danforth ('13), "The myology of Polyodon." At present no group of vertebrates needs a comparative study more than do the teleosts, especially the higher and aberrant forms (Kingsley, '25). Only Esox and Acipenser have been taken from Vetter's ('78) work, because they are the most representative forms. The designations used by Allis were employed as a basis, unless otherwise indicated, because Scom-

ABBREVIATIONS IN TABLE

*A.Pro.Hymd.*, anterior part of m. protractor hyomandibularis
*Br.Dep.*, mm. branchi-depressores
*Br.Lev.*, mm. branchi-levatores
*Br.Ret.Inf.*, mm. branchi-retractores inferioris
*Br.Ret.Sup.*, mm. branchi-retractores superioris
*Con.Ph.*, m. constrictor pharyngis
*Con.Sup.*, m. constrictor superficialis
*Cor.Arc.*, mm. coraco-arcuales
*Dep.Br.Steg.*, mm. depressores branchiostegorum
*Dep.Op.*, m. depressor opercularis
*Dep.Tym.*, mm. depressores tympani
*Hyo.Br.*, m. hyobranchialis
*Hyo.Cl.*, m. hyoclavicularis
*Hyo.Pect.*, m. hyopectoralis
*Int.Arc.Vent.*, mm. interarcuales ventrales
*Lev.Br.Steg.*, mm levatores-branchiostegorum
*Lev.Tym.*, mm. levatores tympani
*Ma.B.*, m. masto-branchialis
*Myl.*, m. mylohyoideus
*N.*, nervus
*O.*, occipital nerve
*P.Ret.*, posterior part of m. retractor hyomandibularis
*Ph.Arc.*, mm. pharyngo-arcuales
*Ph.Tr.*, m. pharyngeus transversus
*Pro.Hymd.*, m. protractor hyomandibularis
*Pty.*, m. pterygoideus
*Rect.Ab.*, m. rectus abdominis
*Ret.Hymd.*, m. retractor hyomandibularis
*Ret.Hyo.*, m. retractor hyoideus
*S.*, spinal nerve
*St.Hyo.*, m. sternohyoideus
*Tr.Dor.*, m. transversus dorsalis
*Typ.*, typical

| Pisces | | Polyodon-spathula | | Acipenser-sturio | | Amia-calva | | Amiurus-catus | | Esox-lucius | | Perca-fluviatilis | | Scomber-scomber | | Opsanus-tau | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group | Author | Danforth '13 | N | Vetter '78 | N | Allis '97 | N | McMurrich '84 | N | Vetter '78 | N | Owen '66 | N | Allis '03 | N | Pankratz '27 | N |
| Eye Muscles. | Three Recti | typ | III | | | typ | III | | | | | | | typ | III | typ | III |
| | Lateral Rectus | + | VI | | | + | VI | | | | | | | + | VI | + | VI |
| | Superior Oblique | + | IV | | | + | IV | | | | | | | + | IV | + | IV |
| | Inferior Oblique | + | III | | | + | III | | | | | | | + | III | + | III |
| Mandibular. | Intermandibularis | — | | Myl. | V<br>VII | + | V<br>VII | + | V<br>VII | + | V<br>VII | + | V<br>VII | + | V<br>VII | — | |
| | Branchiomandibularis | + | | + | | + | 1,2<br>Sp | — | | — | | — | | — | | — | |
| | Geniohyoideus | + | V<br>VII | Con sup 1 - 5 | V<br>VII | + | V<br>VII | + | VII | + | V<br>VII | + | V<br>VII | + | V<br>VII | + | V<br>VII |
| Hyoid. | Hyohyoideus | — | | | | + | V<br>VII | + | VII | + | VII | Lev. Br Steg.<br>Dep. Br Steg | V<br>V | + (2) | V<br>VII | Lev. Br. Steg.<br>Dep. Br. Steg. | V<br>VII |
| | Pharyngo-hyoideus | — | | — | | — | | Hyo Br | IX<br>X | Ph. Arc | X | + | ? | + | X | + | X |
| | Sterno-hyoideus | Cor Arc-Hyo. Cl | X<br>Sp | Cor Arc. pr. a | 1s | + | 1,2<br>3,4,5 | Hyo Pect | 1,2<br>3,4 | + (2) | 1,2<br>Sp | Ret Hyo | 1,2<br>Sp | + | Sp | St Hyo-Rect Ab | 1s |
| Pharyngeal. | Pharyngo-clavicularis-Ext | + (2) | X | — | | + | X | + | IX | + | X | Br Ret Inf | X | + | X | + | X |
| | Pharyngo-clavicularis-Int | or + (3) | .. | — | | + | X | | X | + | X | Br Ret. Sup | X | + | X | + | X |
| | Constrictor Esophagei | + | | — | | Con Ph | | — | | Ph. Tr. | X | Ph Tr | X | + | | + | X |
| Dorsal Mandibular. | Adductor Mandibularis | + (2) | V | + | V | + (3) | V | + | V | + (3) | V | + (3) | V | + (3) | V | Pty. (2)<br>+ | X<br>V |
| | †Adductor Tentaculi | — | | — | | — • | | + | V | — | | — | | — | | — | |
| | †Levator Premaxillaris | — | | — | | — | | — | | — | | — | | — | | + | V |
| | Levator Maxillaris-Sup. | — | | — | | + | V | — | | — | | — | | — | | — | |
| | Adductor Hyomandibular | P. Ret Hymd | V<br>VII | Ret Hymd | V<br>VII | + | VII | + | VII | + | VII | + • | VII | + | V<br>VII | + | V<br>VII |
| Opercular | Dilator Opercularis | Pro Hymd | V | Pro. Hymd | V | + | V | + | V | + | V | + | V | + | V<br>VII | + | V<br>VII |
| | Levator Opercularis | Op. | VII | Op | VII | + | VII | + | VII | + | VII | + | V | + | VII | + | VII |
| | Adductor Opercularis | Ret. Hymd | VII | Ret. Hymd | VII | + | VII | + | VII | + | VII | Dep Op | V | + | VII | + | VII |
| Palatine | Levator Arcus Palatini | A Pro. Hymd | V | Pro. Hymd | V | + | V | + | V | + ? | V | Lev. Tym | ? | + | V<br>VII | + | VII |
| | Adductor Arcus Palatini | Ret. Hymd | V<br>VII | Ret Hymd | VII | — | | + | VII | + ? | VII | Dep Tym | | + | V<br>VII | + | V<br>VII |
| Branchial Dorsal | Interbranchialis | — | | + | IX<br>X | — | | — | | — | | — | | + | | — | |
| | Levator Arcuum Branchialis | + (4) | IX<br>X | + (5) | IX<br>X | + (5) | IX<br>X | + | IX<br>X | + (3) | IX<br>X | Br Lev. (4) | IX<br>X | + (7) | IX<br>X | + (5) | IX<br>X |
| | Interarcuales Dorsalis | — | | — | | — | | — | | — | | + ? | | + (3) | X | — | |
| | Oblique Dorsalis | — | | — | | + (3) | X | + | IX<br>X | + (3) | X | Ma B ? | X | + (3) | X | + (2) | IX<br>X |
| | Transversus Dorsalis Ant. | Tr Dor | | — | | + | X | + | IX | + | ? | + • | X | + | X | + | X |
| | Transversus Dorsalis Post. | | | — | | + | X | | X | | | | | + | X | + | X |
| | Retractor Arcuum Br. | — | | — | | + | X | — | | | | + • | X | + | X | + | X |
| | Adductor Arcuum Br | — | | + (3) | X | + (2) | X | — | | — | | Br. Dep | | + (2) | X | — | |
| Ventral | Oblique Ventralis | — | | — | | + (3) | X | + | X | Ph. Arc (2) | X | + (4) | | + (2) | X | Ph Arc (2) | X |
| | Transversus Ventralis Ant. | + | X | Int Arc Vent (5) | X | + | X | + | IX | + (4) | X | + | | + | X | + | X |
| | Transversus Ventralis Post. | | | | | + | X | | X | + | IX<br>X | + | | + | X | + | X |
| | Trapezius | + | | — | | | | + | 1s | + | 4,2<br>3,1 | + | 1s | — | | + | [illegible] |

† = According to other Authors.

• = According to Vetter.

ber is the nearest related teleost that has been described; and many of Allis' terms are based on the classical investigation done by Vetter ('78). Where the same name was not used by the various investigators, an attempt was made to find its homologue according to structure, nerve supply, and function.

## METHODS AND MATERIAL

The heads of adult toadfish varying from 10 to 20 cm. in length were dissected, under the binocular when necessary. The specimens were macerated in dilute nitric acid, which facilitates dissection of even the very small nerves, since the acid dissolves the connective-tissue sheaths. A number of heads of various sizes were also freed of all the muscles and connective tissue, and the skulls studied for origins and insertions of the muscles described. In order to clean the skulls, they were either boiled in a weak sodium-carbonate solution or allowed to macerate in tap-water.

## DESCRIPTION AND DISCUSSION OF THE CRANIAL MUSCULATURE

For descriptive purposes, the muscles are grouped under the following large divisions: orbital, hyomandibular, opercular, branchial, and pharyngeal groups.

### 1. *The eye-muscle group*

The eyeball of Opsanus tau is large, the orbit long, and correlated with it are long and well-developed eye muscles. The number and arrangement of the muscles conforms essentially to the teleost type, as described by Allis ('03) in his work on Scomber scomber.

*a. Mm. obliqui inferioris et superioris.* The two oblique muscles arise close together at the anterior part of the orbit, from the interorbital septum, at the junction of the ethmoid, vomer, and parasphenoid, posterior to the entrance of the nervus olfactorius into its ethmoid canal. The m. obliquus superioris passes lateralward superior to the nervus olfactorius, which lies free in the orbit for some distance. It is inserted in the bulbar fascia medial to the insertion of the m. rectus superioris, and is supplied by two small twigs from the long slender nervus trochlearis. The m. obliquus inferioris likewise passes laterally to insert near the m. rectus inferioris. It is supplied by a slender branch from the inferior division of the nervus oculomotorius, which passes forward under the medialis and recti inferior and ventral to the nervus olfactorius.

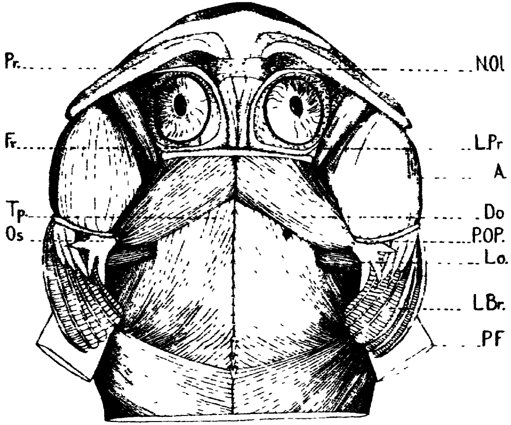
*b. Mm. recti medialis et lateralis et superioris et inferioris.* The four recti muscles arise from a small area about the union of the interorbital septum, the basisphenoid, and the parasphenoid. Their origin is ventral to the nervus opticus which passes to the eyeball over the superior rectus and lateral to the medial rectus. They all become wide and tendinous, to insert on the fascia of the eyeball posterior to the edge of the cornea. The medial and inferior recti are supplied by branches from the inferior division of the oculomotor, and the superior rectus by the small upper division of the oculomotor. The lateral rectus is supplied by the abducens which penetrates its proximal third by two or three twigs.

### 2. *The hyomandibular group*

*a. M. adductor mandibularis.* The m. adductor mandibularis is the most conspicuous dorsal muscle in the toadfish. It arises from the anterior surface of the preoperculum, and the anteroventral margin of the frontal bone. The muscle appears to be composed of three well-fused parts and forms a heavy tendinous insertion on the posterior and inner surface of the triangular process on the articular (*A.*, figs. 1 and 3). It is supplied by four or five small branches from the nervus maxillaris inferioris (*Inf.M.*, fig. 11). The well-developed adductor of the mandible opposed by the large flat m. geniohyoideus, functions as a very powerful adductor of the mandible. The structure of this group of muscles is well correlated with the voracious and carnivorous habits of the toadfish.

## ABBREVIATIONS IN ALL FIGURES

*A.*, m. adductor mandibularis
*Aap.*, m. adductor arcus palatini
*Ah.*, m. adductor hyomandibularis
*Ao.*, m. adductor opercularis
*Ar.*, articular
*B.B.*, basibranchial
*B.H.*, basihyal cartilage
*B.R.G.*, branchiostegal rays
*Buc.f.1*, n. ramus buccalis facialis medialis
*Buc.f.2*, n. ramus buccalis facialis lateralis
*C.*, cerebrum
*CB.*, cerebellum
*C.B.3*, third ceratobranchial
*C.B.4*, fourth ceratobranchial
*C.E.*, m. constrictor esophagei
*C.H.*, ceratohyal (hyoid)
*Cl.*, cleithrum (clavicle of old authors)
*Com.VII–X*, commissure between VII and X
*D.*, dentary
*D.Br.*, mm. depressores branchiostegorum
*Do.*, m. dilator opercularis
*Fr.*, frontal
*F.Rec.*, recurrens facialis
*G.*, gills
*Gh.*, m. geniohyoideus
*H.B.1*, hypobranchial (first)
*Hh.*, m. hyohyoideus
*HMD.*, hyomandibular
*Hmf.*, nervus hyomandibularis
*Inf.M.*, nervus maxillaris inferioris
*Labe-a*, m. levator arcus branchialis externus anterioris
*Labe-m.*, m. levator arcus branchialis externus medialis
*Labe-p*, m. levator arcus branchialis externus posterioris
*Labi-a*, m. levator arcus branchialis internus anterioris
*Labi-p*, m. levator arcus branchialis internus posterioris
*L.Br.*, mm. levatores branchiostegorum
*Ll.V.*, nervus lineae lateralis vagi
*L.O.*, m. levator opercularis
*L.Pr.*, m. levator premaxillaris
*Md.*, medulla oblongata
*M ef.*, n. ramus mandibularis externus facialis
*Mx.*, maxilla
*N.Ac.*, nervus acusticus
*N.GL.*, nervus glossopharyngeus
*N.OL.*, nervus olfactorius
*O.B.*, bulbus olfactorius
*Obd.*, m. obliquus dorsalis superficialis
*Op.*, operculum
*Op.f.*, ramus ophthalmicus superficialis
*Op.L.*, lobus opticus
*Op.t.*, ramus ophthalmicus trigeminus
*OS.*, opercular spine
*Ov.1-2*, m. obliquus ventralis 1-2
*P.B.1*, pharyngo-branchialis (first)
*Pce.*, m. pharyngo-clavicularis externus
*Pci.*, m. pharyngo-clavicularis internus
*Pv.F.*, pelvic fin
*P.f.*, nervus palatinus facialis
*P.F.*, pectoral fin
*P.Gl.*, nervus palatinus glossopharyngeus
*Ph.*, m. pharyngo-hyoideus
*P.OP.*, preoperculum
*Pr.*, premaxillaris
*P.S.*, parasphenoid
*Pt.a.*, m. pterygoideus anterioris
*Pt.p.*, m. pterygoideus posterioris
*Q.*, quadrate
*R.A.*, m. rectus abdominis
*Rbad.*, m. retractor branchialium arcuum dorsalis
*S.*, skull
*Sh.*, m. sternohyoideus
*Tda.*, m. transversus dorsalis anterioris
*Tdp.*, m. transversus dorsalis posterioris
*Te.V.*, nervus temporalis vagi
*Tp.*, m. trapezius
*Tr.R.*, nervi trematici vagi
*Tva.*, m. transversus ventralis anterioris
*Tvp.*, m. transversus ventralis posterioris
*U.L.*, upper lip
*V.*, vertebra
*VO.*, vomer
*II.*, nervus opticus
*III.*, nervus oculomotorius
*IV.*, nervus trochlearis
*VI.*, nervus abducens



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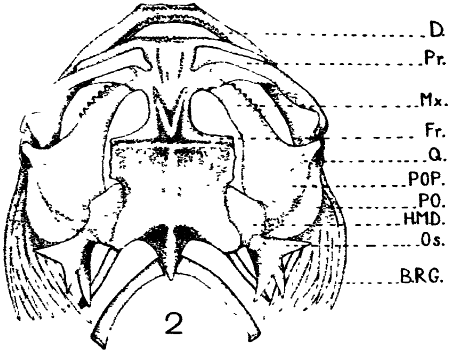

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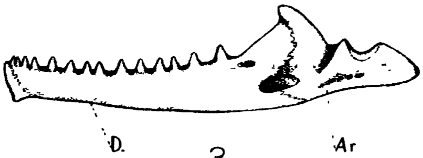

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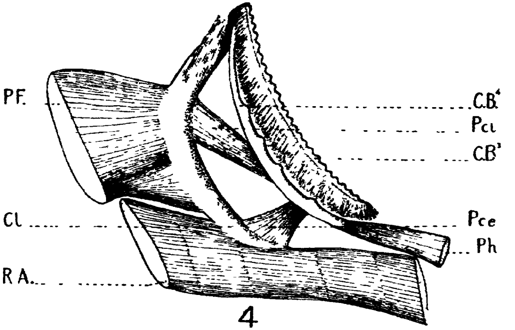

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Fig. 1 Dorsal view of the head, showing the superficial musculature.
Fig. 2 Dorsal view of skull (modified from Clapp).
Fig 3 Mesial view of the right mandible.
Fig. 4 Lateral view of the right cleithrum and part of the fourth and third branchial arches with the associated muscles.

*b. Mm. pterygoidei.* The m. pterygoideus anterioris lies medial to the anterior portion of the m. levator arcus palatini and the origin of the m. levator premaxillaris. It is very large at its origin, arising from most of the ventral surface of the frontal bone and forming the posterior wall of the orbit. Toward its insertion it becomes small and tendinous to insert on the mesial surface of the articular ventral to the insertion of the m. adductor mandibularis (*Pt.a.*, figs. 3 and 7). It is supplied by a branch from the nervus maxillaris inferioris.

The m. pterygoideus posterioris (*Pt.p.*, fig. 7) arises from the anterior surface of the hyomandibular bone and the ventral surface of the postorbital ossification. It is a thin triangular muscle which becomes totally tendinous at its insertion, with the anterior pterygoid on the articular. It receives its nerve supply from the nervus maxillaris inferioris.

The two mm. pterygoidei are possibly homologous to the deeper parts of the m. adductor mandibularis, as described by previous writers. They aid the m. adductor mandibularis in powerful adduction of the mandible.

*c. M. levator premaxillaris.* The m. levator premaxillaris is a long, narrow muscle arising from the frontal, just medial to the origin of the anteromedial fibers of the m. adductor mandibularis. At its origin it is a wide, flat tendon and it inserts on the superior surface of the premaxillaris about one-third from its lateral extremity (*L.Pr.*, fig. 1). It receives two or three small rami from the nervus maxillaris inferioris. The levator draws the corners of the mouth upward and backward.

*d. Mm. arcus palatini.* Medial to the origin of the m. adductor mandibularis the m. levator arcus palatini arises from the ventral surface of the frontal and from the postorbital ossification. It is a flat, triangular muscle with the base dorsal and its apex inserted on the anterior surface of the dorsal end of the hyomandibular and the metapterygoid. The nervus maxillaris inferioris supplies this muscle with two or three small rami. It acts as an elevator of the hyomandibular and

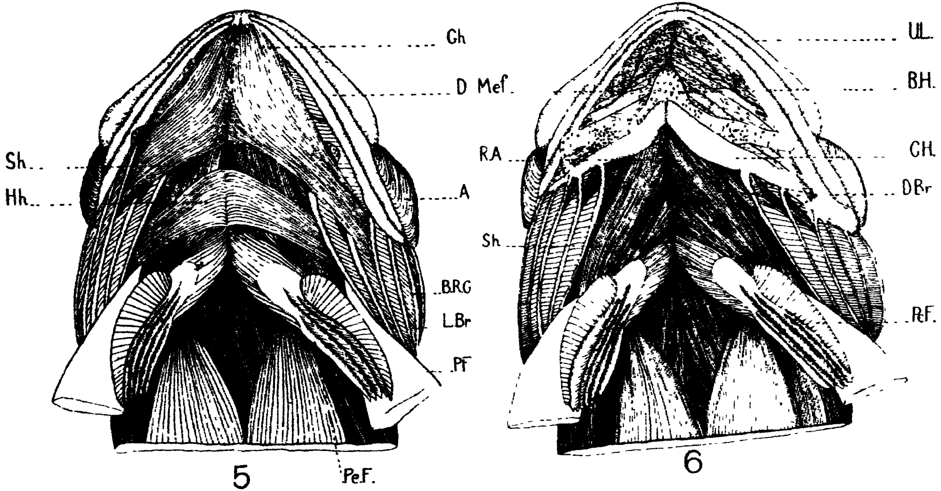


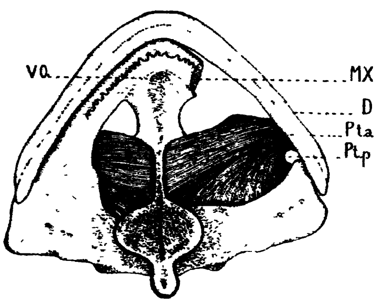
Fig. 5 Ventral view of the superficial layer of muscles.
Fig. 6 Ventral view of the deeper layer of muscles.

the metapterygoid, thus increasing the width of the palatine arch.

The adductor hyomandibularis et adductor arcus palatini are one continuous muscle. They arise from the lateral surface of the parasphenoid and the vomer, and form a flat, arched muscle in the roof of the mouth (*Aap.Ah.*, fig. 8). A heavy fascia covers them dorsally and the thick and tough buccal lining ventrally. The fibers pass laterally to insert on the inner surface of the hyomandibular, maxillary, and the mesial surface of the whole palatine arch. A portion of the m. adductor arcus palatini forms the ventral floor of the orbit. The nerve supply to this pair of muscles is from the nervus hyomandibularis. Their function is to adduct the palatine arch by depressing the hyomandibular and maxillary bones.

*e. M. geniohyoideus.* The most conspicuous muscle on the ventral surface is the long, flat m. geniohyoideus that superficially covers the floor of the buccal cavity (*Gh.*, fig. 5). Its fibers originate from most of the anterior surface of the ceratohyal (hyoid, Kingsley, '25) and the base of the third branchiostegal ray. They pass medially and anteriorly to insert in a median aponeurotic band and the posterior surface of the dentary near the symphysis menti. The lateral part of the muscle near its origin is somewhat confined by a cartilaginous process on the ceratohyal (figs. 5 and 6). This leaves a narrow area between the m. geniohyoideus and the mandible where the buccal floor is formed by a heavy fascia with a few transverse muscle fibers, suggesting a m. mylohyoideus. The m. geniohyoideus is supplied by a large branch from the nervus mandibularis facialis externus (*McF.*, fig. 6). When the hyoid bone is fixed by its other muscles, the m. geniohyoideus functions as the depressor of the mandible. When the mandible is fixed or adducted, it forcefully depresses the hyoid apparatus as in regurgitation of food and expulsion of water.

*f. M. hyohyoideus.* The m. hyohyoideus in the toadfish is a long, flat superficial muscle (*Hh.*, fig. 5). It arises from a



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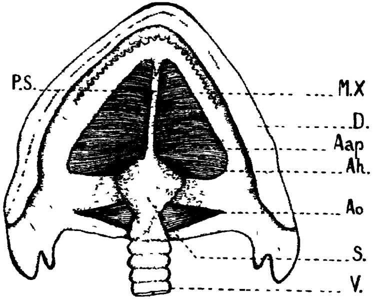

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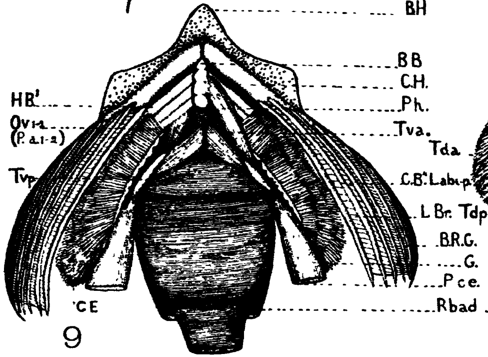

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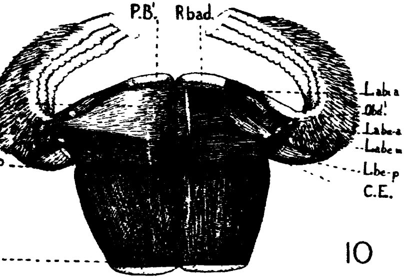

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Fig. 7 Ventral view of the mm. pterygoidei with mandible intact.

Fig. 8 Ventral view of the buccal roof (palatine arch), showing the m. adductor arcus palatine, m. adductor hyomandibularis, and m. adductor opercularis.

Fig. 9 Ventral view of the branchial apparatus.

Fig. 10 Dorsal view of the branchial apparatus.

median aponeurosis, common to both, which extends forward to the first basibranchial (copula, Gegenbaur, '98). It passes posterolaterally to insert on the medial surface of the ventral branchiostegal ray at the point of its greatest curvature. A branch from the hyomandibular nerve ramifies over its middle ventral surface. The hyohyoideus in the toadfish functions as the most powerful depressor of the branchiostegal rays.

### 3. *The opercular group*

*a. M. dilator opercularis.* The m. dilator opercularis is a large triangular muscle which lies in the dorsal temporal groove (*Do.*, fig. 1). It also takes origin from a dorsomedial and transverse crest of the frontal, from the postorbital, squamosal, and the dorsal groove in the hyomandibular bone. The fibers converge and pass posterolaterally to insert on the opercular spine superior to the articulation of the operculum with the hyomandibularis. It is supplied by small branches from the nervus maxillaris inferioris.

*b. M. levator opercularis.* The m. levator opercularis is a much smaller conical muscle (*Lo.*, fig. 1), which arises from the posterodorsal surface and crest of the squamosal bone. As in Scomber, it is contiguous with the dilator at its origin, and its anterior fibers run parallel to, and in contact with, the posterior fibers of the former. It is supplied by a small superior branch of the nervus hyomandibularis. The two muscles abduct and elevate the operculum, thus increasing the capacity of the branchial cavity. This pair is opposed by the m. adductor opercularis and the ventral hyoid group.

*c. M. adductor opercularis.* The m. adductor opercularis (*Ah*, fig. 8) is a triangular muscle arising from portions of the lateral surface of the squamosal, intercalar, petrosal, and lateral occipital bones. Its anterior fibers originate just posterior to the foramen of the facial nerve and pass posterolaterally immediately posterior to the branchial levators. The apex of the triangular muscle inserts on the inner surface of the operculum. It is supplied by the nervus hyomandibularis and acts as a strong depressor of the operculum.

### 4. *The branchial group*

*a. M. depressores branchiostegorum.* In the second ventral layer there are three pairs of small triangular muscles which

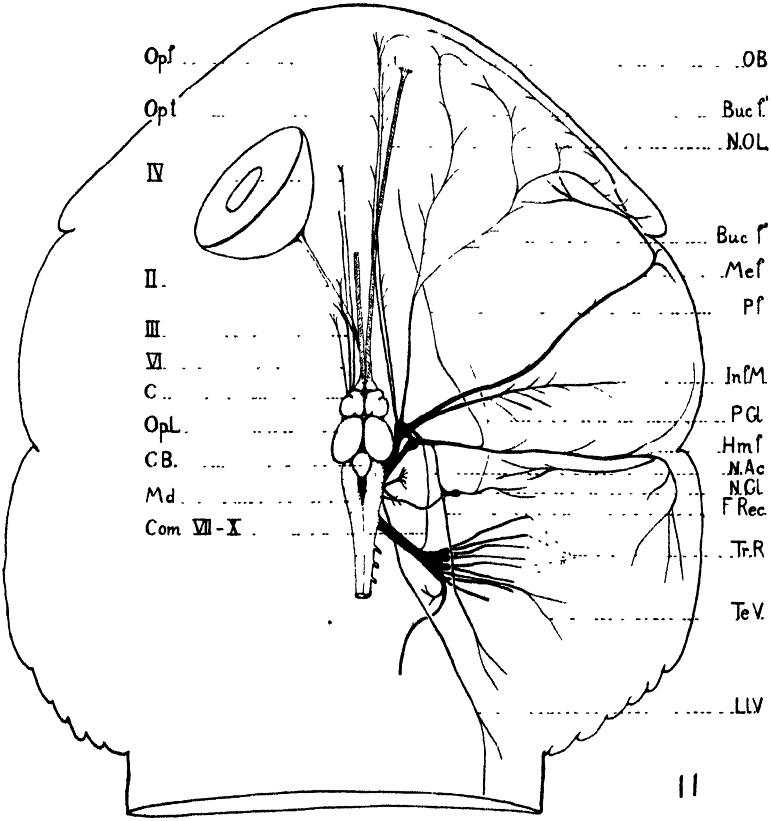

Fig. 11 Dorsal optical view of the brain and the cranial nerves.

connect the ceratohyal and the bases of the branchiostegal rays. The medial is the largest and arises from the posteromedial surface of the ceratohyal and inserts on the medial surface of the proximal portion of the first branchiostegal ray (*D.Br.*, fig. 6). The other two parts have their origin from the more distal part of the ceratohyal and the lateral surface of first and second rays, and insert on the medial surfaces of the second and third rays. They are supplied by the nervus hyomandibularis. This group act as depressors of the branchiostegal rays (figs. 5 and 6); hence the name depressores branchiostegorum (Owen, '66) is appropriate. Owen ('66) describes this group of muscles in Perca fluviatilis. These muscles are not mentioned by other authors and are apparently absent in the more generalized fishes. This group with the mm. levatores branchiostegorum regulate the capacity of the branchial chamber. Since the toadfish is a sluggish bottom feeder, this muscular arrangement makes possible forceful expulsions of water under asphyxial conditions.

*b. M. levatores branchiostegorum.* The muscles of the branchiostegal rays are well developed. As in Scomber, the toadfish usually has seven branchiostegal rays (*B.R.G.*, fig. 5). These rays are firmly bound together by a heavy fascia, in whose inner layer lies embedded a layer of thin oblique muscle fibers. These muscle fibers elevate and draw the rays together, thus aiding the mm. depressores in regulating the size of the branchial chamber (*L.Br.*, fig. 1). To the writer's knowledge, similar muscles have been described only in Perca by Owen ('66). He calls them the levatores branchiostegorum, which is used by the writer. These muscles receive small rami from the nervus hyomandibularis.

*c. Mm. levatores arcuum branchialium.* The mm. levatores arcuum branchialium consist of two interni and three externi (*Labi, a, p, Labe, a, m, p,* fig. 10). They arise from the base of the skull, from the ventral surface of the petrosal, intercalar, and squamosal. The posterior externus inserts on the fourth branchial arch, which has no gills, and is nearly twice as large as the others. The third and second arches have only one levator, while the first arch has a separate muscle. The first two externi insert on the epibranchial cartilages (suprapharyngeal processes of Allis, '03), immediately proximal to the gills. The third externus inserts on the epibranchial cartilage opposite the insertion of the m. pharyngoclavicularis. These muscles raise the gill arches and draw them slightly toward the median line. The two internal levators arise immediately medial to the three external muscles, and pass ventrally to insert on the dorsal surface of the three pharyngobranchial cartilages near the median line (fig. 10). Allis ('03) calls these cartilages the infrapharyngo branchials.

The nervus glossopharyngeus passes laterally between the two internal levators, its ganglion (fig. 11) lying immediately lateral to the anterior internal and medial to the anterior and middle external levators. The five levators are supplied by the branches of the ninth and tenth nerves of their respective arches. The anterior externus and internus are supplied by the nervus glossopharyngeus.

*d. Mm. obliqui dorsales.* The two dorsal oblique muscles (mm. adductores arcuum branchialium) have a common origin from the posterior surface of the second pharyngobranchial cartilage between the insertion of the retractor arcuum branchialis dorsalis and the anterior levator arcus branchialis internus. The most dorsal is the larger and inserts on the epibranchial cartilage of the fourth medially to the external levator (*Obd.*, fig. 10). The deeper and smaller oblique is covered by the former and inserts on the epibranchial cartilage of the third arch laterally to the mm. retractores arcuum branchialium dorsales and in conjunction with a slip of the m. transversus dorsalis posterioris. They are supplied by rami from the first divisions of the nervus vagus. Their action is primarily to adduct the branchial arches, although apparently only a limited amount of adduction is possible.

*e. Mm. retractores arcuum branchialium dorsales.* The mm. retractores arcuum branchialium dorsales (*Rbad.*, figs. 9 and 10) are paired muscles lying longitudinally and dorsally to the pharynx. They arise by a continuous, tendinous slip from the bodies of the fourth, fifth, and sixth vertebrae. The muscles become wider and pass ventral to the mm. pharyngei transversi dorsales to insert in the roof of the pharynx.

Their insertion is on the first three pairs of pharyngo-branchial arches, medial to the insertion of the internal levators and the dorsal oblique muscles. Small rami from the fourth division of the nervus vagus pierce the anterior portion. They function as a powerful retractor of the branchial arches, causing the individual arches to separate, thus opposing the dorsal oblique muscles.

### 5. *The pharyngeal group*

*a. Mm. pharyngei transversi.* Most generalized fishes have a single muscle mass in the dorsal wall of the pharynx (m. pharyngeus transversus ventralis, auctorum). In Scomber scomber, however, these muscle masses are more specialized and divide into an anterior and a posterior muscle. This condition also exists in Opsanus tau. The m. pharyngeus transversus dorsalis anterioris (*Tda.,* fig. 10), the larger of the two dorsal muscles, arises in common with its mate from a median raphé. It passes laterally between the two internal levators of the branchial arches to insert with the second levator arcus branchialis externus on the epibranchial cartilages of the second and third arches. The m. pharyngeus transversus dorsalis posterioris (*Tdp.,* fig. 10) is much smaller and is composed of two minor slips arising in the median common raphé and inserts on the epibranchial cartilages of the third and fourth branchial arches. Their nerve supply is from the rami of the nervus vagus supplying the arches related to each muscle. They act, as Danforth ('13) says of Polyodon, as constrictors of the pharynx.

The mm. pharyngei transversi ventrales originate from the median aponeurosis and the ventral surfaces of the hypobranchials of the third and fourth arches. The anterior passes posterolaterally to insert on the medial surface of the ceratobranchial of the fourth arch near the insertion of the pharyngohyoideus. This muscle appears to be the muscle which Vetter ('78) describes as the M. pharyngeus transversus (*Tva.Tvp.,* fig. 9). The posterior muscle is wide and flat, having a very extensive insertion along the mesial surface of the fourth ceratobranchial arch. Both muscles are supplied by branches from the fourth ramus of the nervus vagus. They function as constrictors of the pharynx, while the anterior also slightly pulls the pharynx forward.

*b. Mm. pharyngoclaviculares.* In the toadfish, as in Scomber scomber, two muscular slips connect the pectoral girdle with the pharynx. The largest, the m. pharyngoclavicularis internus (*Pci.*, fig. 4) originates from the anterior surface of the cleithrum (clavicle of older writers) opposite the pelvic fin. It passes medially and forward to insert on the ventral curvature of the fifth ceratobranchial opposite the origin of the pharyngohyoideus muscle.

The M. pharyngoclavicularis externus (*Pce.*, figs. 4 and 9) arises from the anterior surface of the cleithrum, just opposite the pectoral fin and passes forward somewhat dorsomedially to insert on the posterior surface of the fifth ceratobranchial arch near the internus. They are both supplied by branches from the pharyngeal root of the nervus vagus (fig. 11). Their most important action is to draw the pharyngeal and the whole branchial apparatus backward and downward.

*c. Mm. pharyngo-abdomini.* Due to the jugular position of the pelvic fins, the ventral musculature is somewhat different from that in any of the forms tabulated on the chart. The superficial layer is a cephalad extension of the ventral abdominal musculature which continues over the cleithrum to attach on the ventral surfaces of the second, third, and fourth basibranchials. A band of fascia separates the two divisions of the ventral musculature. It shows the definite segmentation of the body musculature. This layer may be called a rectus abdominis (*R.A.*, fig. 4). Maurer ('12) describes a similar arrangement in various teleosts. A deeper layer arises from the anterior surface of the cleithrum and passes forward to insert on the posteroventral surface of the last basibranchial (*Sh.*, fig. 6). This corresponds to the m. sternohyoideus in Scomber, although no sternum is present. This group is supplied by the first spinal nerve. Their usual action is to pull the hyoid bone backward and downward, thus depressing the floor of the mouth. With the hyoid bone fixed, they will slightly pull the cleithrum and pelvic girdle forward.

*d. Mm. interarcuales ventrales.* Lateral to the median ventral aponeurosis and dorsal to the m. stenohyoideus lies the round m. pharyngohyoideus. It arises from the posterior surfaces of the first and second basibranchials (Kingsley, '25) and passes posterolaterally to insert on the anterior surface of the hypobranchial of the fourth arch medially to the insertion of the m. pharyngoclavicularis internus (*Ph.*, figs. 4 and 9). This muscle is supplied by a pharyngeal ramus of the nervus vagus. When the hyoid is fixed, it draws the pharyngeal arch forward; and when the pharynx is fixed, it depresses the buccal floor.

Dorsal to the m. pharyngohyoideus, there are two small oblique muscles (*Ov., 1–2*), called the fourth ventral oblique in Scomber by Allis ('03) and mm. pharyngo-arcuales by Vetter ('78). To the writer the name used by Vetter seems preferable. The larger and most ventral arises from the posterior surface of the second basibranchial and hypobranchial cartilages to insert on the anterior surface of the hypobranchial of the fourth arch dorsal to the m. pharyngohyoideus (*P.a.1*, fig. 9). Inserting with this is the smaller and deeper muscle which arises from the third basibranchial (*P.a.2*, fig. 9). These muscles are supplied by the pharyngeal ramus of the nervus vagus. Their function is like that of the pharyngohyoideus.

*e. M. constrictor esophagei.* The m. constrictor esophagei is well developed in the toadfish. The dorsal portion of the constrictor lies ventral to the mm. retractores arcuum branchialium and the m. transversus dorsalis posterioris. In Amia and Polyodon the constrictor pharyngis (esophagei) is continuous with the transversus posterioris on both dorsal and ventral surfaces. In the toadfish this muscle is continuous only with the m. transversus posterioris ventralis. It is a constrictor attached continuously to the entire inner surface of the fourth branchial arch, with strongly developed connections to the pharyngobranchial and epibranchials of that arch (*C.E.*, figs. 9 and 10). It always presents a well-developed posterior annular portion (fig. 9). It is supplied by small rami from the pharyngeal rami of the nervus vagus. The large constrictor makes possible such forceful regurgitations as the toadfish manifests. All of the preserved specimens have an everted esophagus.

### 6. *Pectoral and pelvic girdle musculature*

Only a very general statement can be made in this paper concerning this group. The description of the m. trapezius is in an unsettled state, and the two girdles need further investigation. As Vetter ('78) has stated, the trapezius in bony fishes is not present as a muscle which passes from the pectoral girdle to the cranium. The trapezius is rather represented by a layer of dorsolateral trunk musculature which inserts on the superior occipital, exoccipital, parietal, and lateral occipital bones (*Tp.*, fig. 1). It does, however, give an attachment to the suprascapular as it passes over that bone. It is supplied by the first spinal nerves. Its action is to elevate the head slightly, but more to aid in the lateral body movements.

The pectoral muscles have been described in such forms as Scomber, Polyodon, and Amiurus in conjunction with the cranial musculature. The author has found that the pectoral muscles are well developed, due to the large fin, and conform very closely to the description given by Allis ('03) of Scomber scomber (figs. 1, 4, 5, and 6).

The pelvic fin is small and lies anteromedially to the pectoral fin. Its musculature is the most complicated in structure of all the fins (Danforth, '13). The pelvic fins and their musculature have been thoroughly described in a large number of forms by Davidoff ('78, '80).

## DISCUSSION

In general, the cranial musculature of the toadfish is like that of Amia and Scomber, although less complex. The definite differences are: no m. intermandibularis; no m. branchiomandibularis; a long conical m. levator premaxillaris is present, supplied by the fifth nerve; seven branchiostegal rays connected by a heavy layer of fascia and oblique muscle fibers; and three pairs of triangular-shaped muscles arising from the ceratohyal and the first two branchiostegals inserting on the proximal medial surface of the first three branchiostegals successively. The latter are called depressores branchiostegorum, as described by Owen ('66) in Perca fluviatilis. Especially well developed are the masticator muscles represented by the prominent cylindrical adductor mandibularis and two triangular-shaped anterior and posterior pterygoideus muscles. The m. levator arcus palatini lies between the two mm. pterygoidei and the m. adductor mandibularis. The buccal roof is formed primarily by the flat arched m. adductor arcus palatini and the m. adductor hyomandibularis. This group is supplied by the fifth-seventh nerve complex. The large eye and long orbit are correlated with well-developed typical eye muscles.

The operculum is abducted by a large dilator and elevated and depressed by two smaller muscles, all supplied by the fifth and seventh nerves.

The gill apparatus is composed of four branchial arches, the fourth without gills and giving origin to the large m. constrictor esophagei. The arches are elevated by two internal and three external levator muscles; they are retracted by a pair of long mm. retractores arcuum branchialium. Two transverse and oblique muscles draw the dorsal portion of the arches together. Ventrally, the gill arches are adducted and the pharynx constricted by the two mm. pharyngo-arcuales, m. pharyngohyoideus, and the mm. transverse. The branchial and pharyngeal muscles are supplied by the ninth and tenth nerves.

The ventral segmented m. rectus abdominis and the smaller m. sternohyoideus, separated by a median aponeurosis connect the cleithrum and the pelvic girdle with the hyoid bone. These muscles oppose the large m. geniohyoideus in depressing the hyoid bone and the buccal floor. The fourth branchial arch is connected with the cleithrum by a mm. pharyngoclavicularis internus et externus.

The pectoral fins are large and their muscles well developed, while the jugular pelvic fins are small and their musculature complex. The mandibular musculature, as a whole, is well adapted to the voracious and carnivorous habits of the toadfish. The branchial mechanism is adapted for a high degree of efficiency in the utilization of water with a low oxygen content, to which individuals of this species are often subjected. The branchial chamber is large and capable of considerable dilation, due to the length and flexibility of the seven branchiostegal rays which have fascial connections with the operculum and a group of dilators and compressors (mm. levatores et depressores branchiostegorum). The opercular aperture is relatively small and entirely posterior, so that the water during expiration flows not only through the gill slits, but also washes along the more posterior gills on its way out; in this way there is secured a maximal exposure of the water to the respiratory surfaces.

## SUMMARY

1. The cranial musculature of the toadfish is more specialized than any of the teleosts which have been previously described.

2. The eyeball is large and has long typical muscles. No intermandibularis or branchiomandibularis is present. A levator premaxillaris, supplied by the fifth nerve and not described before, is present.

3. This species has seven branchiostegal rays connected by a heavy fascia and oblique levator muscles; they are depressed by three small adductors; the innervation of the two groups is from the fifth-seventh nerve complex.

4. The gill apparatus is composed of four pairs of branchial arches, the last one without gills. The arches have a complete set of levators, adductors, and retractors supplied by the ninth and tenth nerves.

5. Especially well developed are the masticator muscles represented by a large adductor mandibularis and an anterior and a posterior pterygoid muscle. This group is supplied by the fifth and seventh nerves.

6. The pelvic fins are small and have a jugular position, while the pectoral fins are large. Two narrow muscles connect the cleithrum with the pharynx. A short heavy median raphé joins the medial end of the cleithrum and the pelvic girdle.

7. The ventral segmented rectus abdominis, separated by a median aponeurosis, passes over the cleithrum and attaches to the basibranchials. No sternum is present, but a sternohyoid muscle connects the cleithrum with the branchial arches; these muscles and the hyohyoid muscle oppose the large geniohyoid in depressing the hyoid bone and the buccal floor. A large trapezius muscle is present.

8. The cranial anatomy of the toadfish is well adapted to its carnivorous habits; the structure of its branchial apparatus makes efficient respiration possible under asphyxial conditions.

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# CYTOPLASMIC CONSTITUENTS IN THE DEVELOPING EGG OF DISCOGLOSSUS PICTUS OTTH

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SIXTEEN FIGURES

## AUTHOR'S ABSTRACT

The cytological and histochemical evidence presented in this paper shows the following facts 1) The mitochondria are not directly transformed into yolk They may be concerned in its synthesis in so far as they are a factor in the interacting cytoplasmic system, but there are no visible morphological expressions of this functioning. 2) The accumulations of aequeous substances in the cytoplasm in the form of droplets stainable vitally by neutral red, 'vacuoles,' are the forerunners of the first yolk. During the building up of the yolk the aequeous droplets become more and more dehydrated and lose their capacity for being vitally stained. 3) These aequeous vacuoles give the impregnation results commonly ascribed to the Golgi apparatus and are interpreted as such. Special emphasis is laid on the question of the identification of the Golgi apparatus 4) The fat arises de novo in the cell independently of the mitochondria or the vacuoles. It becomes dispersed throughout the cell among the yolk plates, and the lipoidal content of the latter increases at the end of vitellogenesis, when the fatty globules are intimately pressed in among the yolk plates 5) At a late stage in the growth of the yolk plates there is the sudden appearance of large quantities of glycogen in the perinuclear zone and throughout the cytoplasm

## CONTENTS

Introduction ........ 233
Methods ........ 235
Observations and interpretation ........ 235
  Mitochondria ........ 236
  Fat ........ 238
  Golgi apparatus ........ 240
  Yolk formation ........ 246
  Glycogen ........ 249
Summary of conclusions ........ 249
Bibliography ........ 250

## INTRODUCTION

The question of the changes taking place in the cytoplasm of developing eggs has long occupied cytologists. As more precision is reached in methods of approach, more and more accurate information can be obtained on the complex alteration in the formed elements of the cytoplasm during oogenesis. The origin of the yolk spheres, which form by far the largest part of the cytoplasmic content of the mature egg, is one of the problems which has been the center of much controversy. In some cases the yolk has been described as arising from the mitochondria, as, for example, in the cases reported by Lams, Gadjewska, Konopacki, Margaret Murray, Hirschler, and others. Avel, in the case of the frog, was unable to confirm this. On the other hand, many authors have demonstrated an intimate relationship between the Golgi apparatus in the egg cell and the earliest formation of yolk (Parat, Hibbard and Parat, Parat and Parat, Weiner, Gatenby in some cases, Nath, Brambell, Ludford, Wheeler, Steopoe, etc.). These is, in some cases, question as to the validity of comparing these results, since not all cytologists are agreed as to the cellular structure or structures which can be named Golgi apparatus. It is quite evident that the usual methods for demonstrating the Golgi apparatus, i.e., impregnation by silver nitrate or by osmic acid, can give blackened bodies which are not necessarily all of the same constitution. Mitochondria, more or less normal in form, depending upon the excellence of the fixation, fat-droplets, lipoidal granules, and the true Golgi apparatus can be easily confused after these means of treatment. In still other cases (Harvey, Husain, Guthrie, Gatenby and Woodger in Grantia) the yolk is described as arising directly from the homogeneous cytoplasm without relation to any of the preexisting formed substances.

In the following description of the history of the developing egg from its earliest stages up to the ripe egg ready for fertilization, some of these problems are met and a possible solution of the difficulties is offered.

The material used was Discoglossus pictus Otth, sent from Algeria through the kindness of Mr. M. Rose. The work was done during the time when the author held the Sarah Berliner Fellowship of the American Association of University Women, in the laboratory of Comparative Anatomy and Comparative Histology of the Sorbonne, in Paris. It is a great pleasure to express appreciation for the cordial hospitality of Prof. Paul Wintrebert of that laboratory, and for the helpful advice and criticism of Dr. Maurice Parat.

## METHODS

The technique used in the study of the ovaries was extremely varied. The general fixatives, such as Bouin-Hollande, were good for topographic information, but useless as cytological agents. For the Golgi apparatus the osmic-acid methods of Champy, Benoit, Mann, Kolatschev, were employed, but these gave also impregnation of fat and even of mitochondria. The silver-nitrate impregnation methods of Da Fano and Cajal gave information on the Golgi apparatus, but frequently included mitochondria and always the pigment and prepigment granules. For the mitochondria, the best method was found to be Dietrich as modified by Parat, followed by Altmann's acid fuchsin, and counterstained with toluidin blue as indicated by Kull or by the modification of Volkonsky. Numerous fixatives for specific histochemical tests, such as 10 per cent formol in physiological salt solution for research of fats and related substances, absolute alcohol for glycogen, copper acetate in formol for fatty acids, calcium salicylate for soaps. The most important single method was that of vital examination with or without vital staining in neutral red and Janus green. The interest in this method lies in the light it throws on the sections of fixed material. As a sole method of study it would be quite insufficient, because the cells must be examined entire and the fine points visible in sections of 2 μ thickness, as in the Dietrich-Parat Altmann-Kull material, for example, escape notice. However, for an understanding of the general relations of the formed elements of the cytoplasm and as a means of identification of certain easily confused things like the Golgi apparatus, it is indispensable.

## OBSERVATIONS AND INTERPRETATION

In the youngest observed cells in the ovary, a marked polarized condition is visible. This polarity is apparent in the arrangement of the mitochondria, of the earliest globules of fat, of the Golgi apparatus. Gradually the localized groups of these cell constituents become disseminated more or less evenly around the nucleus as a center, some occupying a more peripheral zone, others a zone adjacent to the nuclear membrane. As growth proceeds the arrangement in strata is gradually replaced by a general diffusion and intermixture of cytoplasmic inclusions. This is accompanied by an increase in quantity of these substances as a result of an absorption of material over the surface of the egg cell and processes of elaboration going on continually within the cytoplasm. The most striking of these additional inclusions is the yolk which in the mature cell literally crams the cytoplasm, and among the platelets of which are found the other cell constituents.

### *Mitochondria*

After Dietrich-Parat technique the mitochondria are most clearly visible in the form of fine filaments. They may also be seen following the osmic-acid fixatives and, in some cases, after silver impregnation. At first these filaments are accumulated in a tangled mass on one side of the nucleus (fig. 1). The rest of the cytoplasm is quite homogeneous. From this position filaments appear to loosen themselves from the mass, or 'Balbiani corpuscle,' and migrate out in bunches (fig. 1). As this process goes on they become very much more numerous. It is quite impossible to say whether new mitochondria appear independently in the apparently homogeneous cytoplasm or whether they continue to be crystallized out in association with already existing mitochondria. In any case, they represent morphologically an increasing accumulation of protein and lipoidal constituents of the cytoplasm.

For some time the more concentrated tangled mass persists on one side of the nucleus, and attached to this are still seen strands of mitochondrial filaments which appear to be migrating out. Ultimately, however, the distribution loses its polarized aspect and the entire cytoplasm becomes filled with filaments. At this time the orientation of the mitochondria becomes distinctly different. They all seem to be radially arranged about the nucleus, perpendicular to the surface of the egg. There appears a slight perinuclear zone entirely free from mitochondria, and outside this space there is a zone where the mitochondria are more concentrated, as if they had been pushed passively away from the nuclear membrane and concentrated in this zone (fig. 2). The orientation of all the mitochondria would seem to show the presence of centrifugal currents in the more fluid protoplasm which leave the mitochondria strewn along their paths. In a stage similar to this the eggs fixed by Champy's fluid show the mitochondria throughout the entire cytoplasm agglomerated in dense masses, leaving wide spaces between free from mitochondria. These correspond with the 'crackled' appearance of the cytoplasm figured by Champy and Gley in young ovocytes of many species. Fixation in another osmic-acid fixative, that of Mann, shows the mitochondria more nearly as they appear after Dietrich-Parat. Levi, in other Amphibia, figures filaments as in Discoglossus. Indication that this is the more normal condition rather than the agglomerated masses is found in examining living eggs. The mitochondria are more highly refringent than the surrounding cytoplasm and therefore visible in the unstained living egg. They never show a 'crackled' appearance in the fresh cells. The general distribution of mitochondria described above persists during the early stages of vitellogenesis and fat migration (fig. 3). For some time the filaments are visible among the yolk plates. In later stages granular elements are found along the yolk plates. These may be chondriomites or they may be appearances due to the great abundance of yolk which necessitates cutting the mitochondria transversely in a very thin section, since they are forced out of a given plane by the platelets of yolk. The egg when full of yolk is opaque, and therefore observations on this point in the living egg are impossible.

The polarized mitochondrial mass visible in young stages, which represents the yolk nucleus or nebenkern or Balbiani corpuscle of various authors, is completely disorganized and disseminated through the cytoplasm in the early stages already described. There is no indication of such a body at the beginning of yolk formation or even much later after the yolk plates are well established and considerably enlarged. But in a still more advanced period there is formed de novo on one side of the nucleus a compact body giving the lipoidal reactions (deep blue after Dietrich, orange-yellow after Ciaccio), and showing on close examination a tangled filamentous structure. This mass appears only after the polarity of the egg has become well established and the nucleus displaced to a position nearer the animal than the vegetal pole. It always occurs on the side of the nucleus nearer the vegetal pole. Its duration is not long; it diffuses laterally and before the egg is fully ripe, while the nuclear membrane is still intact, it has completely disappeared.

Weiner, in Tenegaria and Lithobius, has considered in detail the origin and fate of the yolk nucleus. He finds an intimate connection between it and the Golgi apparatus; therefore, a further discussion of this point will be found at the end of the section on the Golgi apparatus.

In all stages the mitochondria give the lipoidal reactions of Dietrich and Ciaccio. That is, after fixation in Helly's fluid and insolubization by potassium bichromate, they stain orange by sudan III and deep blue by Kultschizky's hematoxylin.

### *Fat*

The successive changes undergone by the fat present in the egg can be best observed after fixation in 10 per cent formol in physiological salt solution followed by staining with sudan III on sections cut with the freezing microtome. In the very smallest eggs there is nothing which gives the characteristic red of sudan III on fats. Very soon, however, one or two globules appear (fig. 4). These increase in number as more and more absorption and elaboration take place, and the fat becomes localized, often polarized, just outside the nuclear membrane (figs. 5 and 6). The fat increases, and from this position on one side of the nucleus it migrates around the nucleus until there is a definite zone containing a large amount of fat just outside the nuclear surface (fig. 7). After the commencement of yolk formation, not before, the fat begins to migrate peripherally (figs. 8 and 9). It should be remembered that, at this time, the Golgi apparatus is widely separated from the fat by practically the entire thickness of the cytoplasm. The large compact groups of fatty globules separate into fine droplets and spread through the midzone of the cytoplasm as indicated in figure 10. Here it has disseminated among the spheres of yolk which have migrated into this position and are rapidly growing. The isolated elements of yolk pushed in from the peripheral part of the cell and the globules of fat migrating out from the central region become intimately associated morphologically in this midzone. Then follow the gradual changes leading to the definitive distribution of the yolk in the ripe egg. Here the cytoplasm is packed with yolk throughout, and between the yolk plates are found numerous minute droplets of fat-like material which blacken with osmic acid and give an orange-red with sudan III after formol. It would appear that these fats correspond to the 'fatty yolk' of Brambell, Gatenby, and others.

The reaction of sudan III on the fat is specific. Fat can be demonstrated also by the osmic fixatives, Champy, B. O. S. U., Mann, and others. In all such preparations the globules visible after sudan III can be seen blackened by the osmic acid. Simple fixation without postosmification blackens fat alone; but in the pieces which have undergone prolonged impregnation with osmic acid there are other structures as well which appear black.

The chemical nature of these fat-globules seems to be very complex. The polarizing microscope on fresh eggs shows the material to be isotropic. This rules out simple ethers of cholesterin and certain lecithins which are known to be birefringent. Sections cut with the freezing microtome of ovaries fixed in 10 per cent formol in physiological salt solution (6/1000) give more exact information as to the composition of these globules. The neutral fats present are largely saturated like stearin and palmitin, since the unsaturated olein gives, according to Mulon's reaction, a deep black color by twenty-four hours' treatment with osmic acid on frozen sections. In this case the color is gray, and only after subsequent treatment by 70 per cent alcohol for twenty-four hours does it give definitely the black reaction. The presence of fatty acids in the complex is indicated by a deep blue stain after Nile-blue sulphate, and red after neutral red (Lorrain Smith). These results with neutral red and Nile blue on fixed sections should not be confused with those obtained by vital staining. The technique of Windaus (treatment with digitonin followed by sulphuric acid) gives a positive pink reaction near the surface of the cell, indicating the presence of cholesterin in that region, but no reaction at the level of the fat-globules.

The position occupied by the fats appears after such fixatives as Bouin, Hollande, and Helly as empty spaces which are entirely visible in the sections.

### *Golgi apparatus*

The two generally accepted types of treatment for the demonstration of the Golgi apparatus are impregnation by silver nitrate and by osmic acid following appropriate fixatives. But these methods are not specific for the Golgi apparatus. After Cajal or Da Fano fixation and subsequent treatment by silver nitrate there are many things blackened. Some of these are undoubtedly mitochondria. This can be determined by comparison with the definite mitochondrial methods. However, after Cajal and Da Fano the mitochondria are not always impregnated, and frequently, when visible, they have undergone serious modification as the result of bad fixation. They may appear as slender blackened filaments or as filaments with swellings and granules along their length; they may show apparent anastomoses; or they may be fragmented into isolated granules. All cells of a single section do not contain impregnated mitochondria, even though there are some cells which do. The method is extremely capricious and the reasons for the success or failure of the impregnation are not known. In addition to the possible mitochondria one always finds the pigment and prepigment granules strongly blackened by silver nitrate. These granules are not easily confused with the other things present because of their extremely minute size. The true Golgi apparatus may be impregnated as well as all these other substances. Here, also, the method is extremely capricious, because a whole block of tissue fails to be impregnated at all, or some few cells may be good and the rest worthless, or practically all cells may show nicely the Golgi bodies. One can never be sure to what extent the Golgi apparatus or the mitochondria will be impregnated. By this method the identification of the Golgi apparatus consists in making as complete an impregnation of the cell as possible, eliminating the structures, such as mitochondria and pigment, which have been previously localized by other methods, and after that the unidentified residue is probably the Golgi apparatus.

The other accepted method for showing the Golgi apparatus, that of osmic-acid impregnation, is not more sure. It consists in fixation in some fixative containing osmic acid, such as Champy, Mann, Kolatchef, Nassonov, and subsequent treatment in 2 per cent osmic acid for a long period—up to several weeks. This may be done at room temperature, but the results are hastened if it is done in an oven at 37°. The very first thing to be blackened by the osmic acid is the fat. Then other cell constituents become blackened, and after a very long time the entire cell is black. This progressive impregnation can be arrested at any point by a few simple experiments to determine the optimum time necessary on a given tissue, or a regressive method may be employed as indicated by Parat and Painlevé. Here the technique consists in a progressive bleaching with potassium permanganate and oxalic acid. The degree of bleaching is entirely dependent upon the wishes of the operator, and there is nothing precise about the method. Most of the fat can be removed from the preparation by treatment for several hours in turpentine, but in these eggs there are often left after such treatment some black bodies which correspond exactly in form and position to fats stainable, under other conditions, by sudan III. The conclusion is reached that some of the fat-like substances may be rendered insoluble in turpentine by fixation and postosmification.

These various methods indicate that one cannot with certainty separate the Golgi apparatus from the mitochondria, on the one hand, or the fat on the other, except in a negative fashion. By the methods of Dietrich-Parat, or Regaud-Tupa, or Champy, one can identify the mitochondria and by subtracting these from those cases of silver-nitrate impregnation, where both mitochondria and Golgi bodies are impregnated, one can approximate what represents the Golgi apparatus; by formol-freezing methods the fat can be localized, and by subtracting what one sees by this means from the total osmic pictures one can likewise approximate the Golgi apparatus.

In the present case of Discoglossus the two methods arrive at the same result—an impregnated Golgi apparatus which undergoes progressive changes in the developing egg. From an original irregular distribution in groups of discrete bodies which appear to migrate from a given point on one side of the nucleus, these elements approach the surface of the egg and take up a position in the peripheral zone of the cytoplasm.

But there exists, in addition to these, a specific method of staining the Golgi apparatus in these eggs. They may be stained vitally by neutral red. That is, the only structures in the egg that can be identified by methods ordinarily in use by the majority of cytologists (Nassonov, Bowen, Gatenby, Brambell, Ludford, Perroncito, Cajal, and others) for demonstrating the Golgi apparatus, i.e., those structures indicated above as the result of the techniques of silver or osmic impregnations, can be put in evidence by vital staining with neutral red, and no other cell constituents, with the possible exception of the pigment, are vitally stained by this means. In addition there is the large amount of evidence of Parat and his collaborators as to the specificity of neutral red for the Golgi apparatus in a variety of cells. The coincidence between the impregnated Golgi apparatus in fixed material and the vacuoles stainable by neutral red in the living cell is astonishing. The word vacuole and the inclusive term 'vacuome,' always used by Parat, which includes the sum of the entire vacuolar system of the cell, are not descriptive terms. As in all questions of nomenclature, the priority rule holds true, and these bodies, long described as vacuoles in plant cells, are no more empty spaces in the cytoplasm than the cell itself, first described by Hooke, is a hollow box of corky material. In the discussion of the formation of the yolk of these eggs the question of the contents of the vacuoles or Golgi elements will be considered.

The ovary of Discoglossus is a material upon which vital staining is accomplished with difficulty. The postvital method of removing the organ and allowing it to stand some time in Ringer's solution saturated with neutral red, which is quite successful on certain tissues, such as ovaries of the perch, gave absolutely no coloration at all. The only staining was after several hours, when the entire cell became permeable because of its death and the nucleus was colored red. After the death of the cell neutral red has no further electivity for the Golgi bodies.

Vital staining was accomplished by injection of neutral red saturated in Ringer's solution. This saturation must be done at a higher temperature than that of the laboratory, and when precipitation occurs after several days of normal temperature, the solution must be reheated. Injections of 2 to 4 cc. of neutral red alternately into the lymph sacs, into the peritoneal cavity, and into the digestive tract by way of the mouth, every day for a period of ten days, gave good results. After this the animal may be killed, the ovary teased apart in Ringer's solution, and the smaller eggs mounted on a slide. By this means the whole egg is examined, and until the stage is reached where the yolk is well formed, the egg is sufficiently transparent so that the whole of the cytoplasm may be studied. Later, the opacity due to the accumulation of yolk renders vital examination of the deeper regions impossible, but the superficial regions can be studied even in the latest stages.

In the young stages the vacuoles colored by neutral red are localized on one side of the nucleus (fig. 11). This general region corresponds exactly with the polarized mitochondria observed in sections. In fact, in the living egg the mitochondria are clearly visible as refringent bodies, closely applied to and winding among the red vacuoles. This relationship is strongly reminiscent of that observed in the egg of Amblystoma by Parat and Painlevé, and also that in the egg of the perch (Hibbard and Parat). As was pointed out in the latter case, the close morphological relation of these two cellular elements may be the source of confusion in determining their chemical composition. Lipoids can always be demonstrated around the vacuoles, but never in them, contrary to the findings of Ciaccio. The source of the lipoids may be in large part the mitochondria, and also there are often accumulations of lipoidal substances in the cytoplasm of the region. Not all the histochemical reagents are good cytological fixatives, and the normal mitochondria, known to be delicate structures, are often deformed. The surface film separating the aqueous vacuolar contents from the non-aqueous colloids of the cytoplasm plays undoubtedly a physical part in the lipoid accumulations.

Weiner, in discussing the yolk nucleus in myriopods, indicates a possibility that the yolk nucleus may be the product of secretory activity of the Golgi apparatus. No observations at all were made upon living eggs. There is a possibility of a confusion in Weiner's work as to the elements he calls Golgi apparatus. He states that they stain by fuchsin and hematoxylin after Helly's fluid even more readily than do the mitochondria. In practically all cases the Golgi bodies are put in evidence with the greatest of difficulty by the most capricious methods. How can they be the same cell constituents if they are so resistant to chemicals which ordinarily destroy Golgi bodies? The yolk nucleus described in these special cases by Weiner is probably not comparable to the polarized Balbiani corpuscle in Discoglossus. It is present in quite different stages of vitellogenesis.

There can be no confusion between the vacuoles and the polarized fat in the youngest eggs, because the fat is located much nearer the nuclear membrane. Also it is impossible to stain fat vitally with neutral red. Fat-globules have been observed repeatedly in cells stained in neutral red, and never has there been the slightest red stain on the fat.

From its original position the mass of vacuoles separates into smaller groups of vacuoles which migrate into other regions of the cytoplasm (fig. 12). They are associated for some time with tangled masses of mitochondria. Gradually these clumps of neutral red vacuoles separate and migrate peripherally, taking up the position just under the surface membrane of the cell where we have already noted the Golgi apparatus after impregnation (fig. 13).

At this point the chemical constitution of the contents of the vacuoles becomes changed and they are transformed into yolk plates. The further history, therefore, of the Golgi apparatus, or vacuome, will be considered under the title of 'yolk formation.'

Before leaving the question of the identity of the neutral red vacuoles as compared with the impregnated Golgi elements, we may note that not only do they coincide morphologically at any given stage so far as we can compare two similar cells, but also that the developmental history of the vacuoles and the Golgi apparatus runs parallel. Bowen gives great weight to the developmental behavior of the Golgi apparatus in identifying it in varying types of cells. The evidence that we are dealing with two manifestations of the same thing is even more conclusive in cells of the same kind. Here the classical Golgi apparatus as determined by impregnation methods is found to correspond strikingly with the neutral-red vacuoles.

### *Yolk formation*

The yolk plates may be seen by a variety of means, since, once formed, the yolk is a cellular constituent which persists after all fixatives. There is no possibility of the confusions which may easily arise in connection with the Golgi bodies or mitochondria which react quite differently under varying conditions.

The earliest yolk in Discoglossus is found in the peripheral region of the cell just under the surface membrane. Figure 8 shows a portion of an egg with young yolk plates which were stained red with Altmann's acid fuchsin. They are strictly limited to the outer regions of the cytoplasm. It should be noted that fat is clearly demonstrated near the nucleus and has not the slightest contact with the yolk-forming zone. Just outside the fat there are clouds which represent the accumulation of mitochondria which were more clearly visible in the cells illustrated in figures 2 and 3. The region, therefore, richest in mitochondria has no relation with the zone of the earliest formation of yolk. There exist, of course, filamentous mitochondria throughout the cytoplasm, but they are not more numerous nor larger where yolk is being formed, nor are there any evidences of changes of mitochondria into yolk. In the Altmann-Kull and Altmann-Volkonsky preparations after fixation in Dietrich-Parat, the mitochondria and the yolk give similar staining reactions. In the peripheral region there are more mitochondria in the form of granules than elsewhere, but this is probably due to the fact that the filaments lie parallel to the surface and there is a greater chance of their being cut transversely here than elsewhere. These minute apparent granules resemble very closely the most minute of the yolk plates, but this is not sufficient evidence for assuming a transformation of one into the other, as indicated by Konopacki. Furthermore, the mitochondria are regularly distributed, while the yolk, when newly formed, appears in clumps.

On the other hand, there is a striking correspondence between the impregnated Golgi apparatus and the developing yolk. In the cell after vital staining (fig. 13), the Golgi bodies are located exclusively in the yolk-forming zone (compare fig. 8). From this region there is rapid migration toward the nucleus, shown in the osmic impregnation illustrated in figure 3. The yolk follows a parallel development. From its first position, as in figure 8, it migrates inward (figs. 9 and 10) and is distributed throughout the cytoplasm. In the impregnations of early stages with silver nitrate, such as that shown in figure 8, the yolk is entirely blackened. In the later stages the yolk is very well preserved and can be seen with or without counterstaining, but is not blackened. In favorable sections there are frequently visible black rims or crescents around the yellowish yolk plates. These crescents are never found in the fully ripe eggs. The conclusion here is that the Golgi bodies are transformed directly into yolk, and as the yolk becomes more and more completely elaborated, it loses its ability to be completely impregnated until only a surface film can be blackened, and finally nothing at all.

The evidence for this statement is very much more conclusive as a result of studies of vital staining. The Golgi elements are clearly stained vitally by neutral red in the early stages, as indicated above. They retain their staining capacity throughout their peripheral migration, but in their subsequent diffusion through the cytoplasm they become less deeply stained. The individual vacuoles are gradually divided and distributed in the cytoplasm, and in this stage they are recognizable as yolk plates. In the eggs containing numerous very small spherical yolk plates, all the yolk is colored a yellowish red in contrast to the deeper red of the Golgi apparatus in its younger stages. The transformation is visible in a complete series of intermediate steps. The yolk grows continually in size and the plates become oval in shape. At this point they are no longer colored entirely by neutral red, even faintly, but there remains on the surface a watery rim or crescent which is distinctly stained by neutral red. The evolution of a single yolk plate as demonstrated by vital staining with neutral red is as shown in figure A.

The chemical composition of the Golgi bodies with their later transformation into yolk is only partially known. The earliest stages are undoubtedly aqueous solutions of colloidal substances, probably protein in nature. They are very fluid at first and therefore extremely difficult to fix. The fact of their stainability by neutral red indicated their essentially aqueous composition, since fats are never stainable vitally by neutral red. The pH of these vacuoles can be roughly determined by the tint assumed by the neutral red, and it is found to be slightly acid.

From the moment that the Golgi apparatus is located at the surface layer of cytoplasm, it begins to grow more concentrated and therefore becomes fixable by other methods than the impregnations by silver nitrate and osmic acid. It is visible in the form of the first yolk plates. After this the gradual changes involved in the growth of the yolk during its migration are indicated by the lessened capacity for staining with neutral red and also for impregnation. During this time of synthesis there is undoubtedly a concentration and dehydration going on in the contents of the vacuole. Such a process has been described in the eggs of the invertebrates Chrysaora and Para centrotus by Parat and by Parat and Parat. As the dehydration progresses the remnants of the aqueous phase cling for a period to the surface of the yolk plate, and it is this which forms the rim or crescent which can be seen by vital staining or by impregnation. When the egg has completely matured, there remains nothing except the pigment which is colored by neutral red.

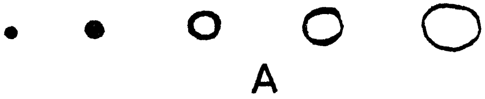

Figure A

During vitellogenesis the largest yolk plates are found in the midzone of the cytoplasm, where they are mingled with the more and more finely divided fat-globules, migrating in a direction away from the nucleus. These various bodies come to lie in intimate contact side by side; this is clearly demonstrated after osmic-acid fixatives. There is an addition of fatty or lipoidal molecules to the yolk at about this time, since the yolk takes a more decidedly orange tint after staining in sudan III.

### *Glycogen*

In the developing eggs of Rana, Konopacki and Konopacka showed in the earliest stages granules of glycogen. These were utilized in the formation of yolk in the cell. In Discoglossus there have been found no evidences of glycogen until an advanced stage in the process of vitellogenesis. Then, quite suddenly, there appears a great amount of glycogen in the perinuclear zone, and scattered in the form of minute granules all through the cytoplasm. It is in this stage that the polarized mass of lipoids is found on one side of the nucleus, as indicated on page 238. After technique for lipoids, this nebenkern gives positive reactions and all the zone around the nucleus out to the boundary of the zone of yolk plates is clear and uncolored; after fixation in absolute alcohol and treatment with iodized gum or Best's carmine, the clear zone is a deep orange or a brilliant rose color, respectively, and the lipoidal nebenkern appears as a light-colored island in the midst of the glycogen.

## SUMMARY OF CONCLUSIONS

The cytological and histochemical evidence herein presented shows, 1) the mitochondria are not directly transformed into yolk. They may be concerned in its synthesis in so far as they are a factor in the interacting cytoplasmic system, but there are no visible morphological expressions of this functioning. 2) The accumulations of aqueous substances in the cytoplasm in the form of droplets stainable vitally by neutral red, 'vacuoles,' are the forerunners of the first yolk. During the building up of the yolk the aqueous droplets become more and more dehydrated and lose their capacity for being vitally stained. 3) These aqueous vacuoles give the impregnation results commonly ascribed to the Golgi apparatus and are interpreted as such. 4) The fat arises de novo in the cell independently of the mitochondria or vacuoles. It becomes dispersed throughout the cell among the yolk plates, and the lipoidal content of the latter increases at the end of vitellogenesis, when the fatty globules are intimately pressed in among the yolk plates. 5) At a late stage in the growth of the yolk plates there is the sudden appearance of large quantities of glycogen in the perinuclear zone and throughout the cytoplasm.

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# PLATES

## EXPLANATION OF PLATES

All figures were drawn with the camera lucida. The scale, in micra, appears on each plate. This scale was drawn from a graduated slide with the camera lucida, using the same set of lenses used for the drawing of the cells and has subsequently undergone the same reduction as the drawings. It will be noted that plate 2 has been reduced more than plate 1—a point to be considered in comparing the eggs.

## PLATE 1

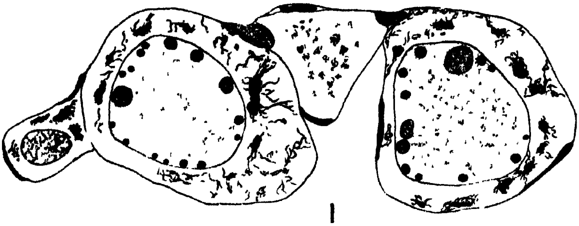
### EXPLANATION OF FIGURES

1 Group of young oocytes. Dietrich-Parat fixation, Altmann-Kull stain. Mitochondria appear as filaments and granules. Compare distribution in smallest cell with that in largest.

2 Older oocyte. Same fixation and stain. Mitochondria distributed throughout, except for perinuclear zone.

3 Still older oocyte. Mann fixation, Altmann's acid-fuchsin stain. Note fat in black, mitochondrial filaments, and clear circles outlining yolk spheres.

4 to 7 Formol fixation, sectioned by freezing microtome, sudan III stain. Note increase in number and change in position of fat-globules here drawn in black, but seen as brick-red in the preparations.



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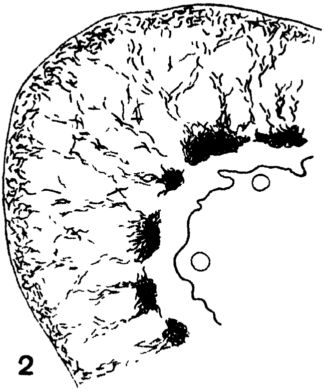

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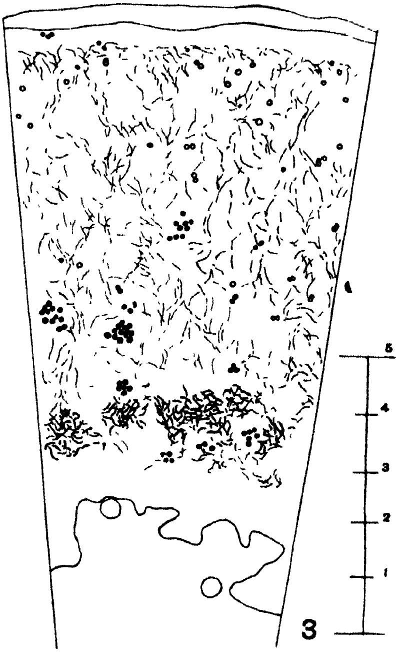

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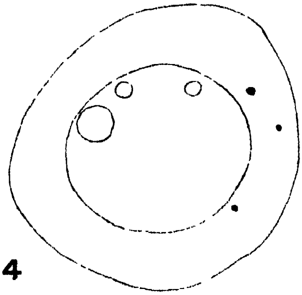

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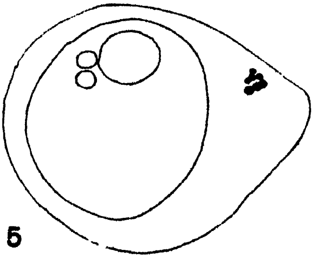

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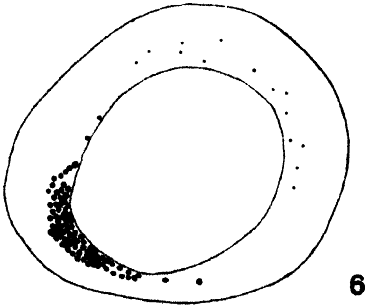

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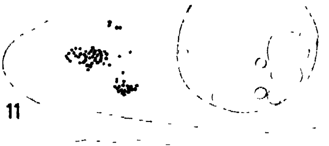
## PLATE 2

### EXPLANATION OF FIGURES

8 to 10 Successive stages in yolk and fat distribution. Champy fixation, Altmann's acid-fuchsin stain. Fat blackened, yolk red (here in simple outline).

11 to 13 Living cells vitally stained with neutral red. Note arrangement of vacuoles and contrast with fat-globules.

14 and 15 Peripheral portions of two living eggs containing yolk. Vitally stained with neutral red.



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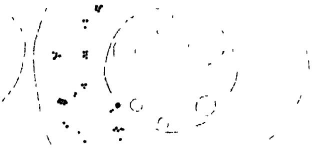

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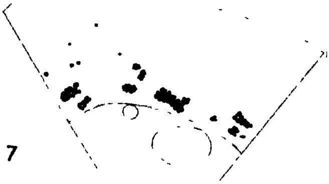

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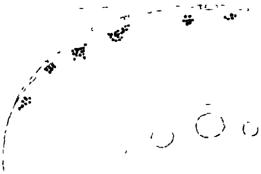

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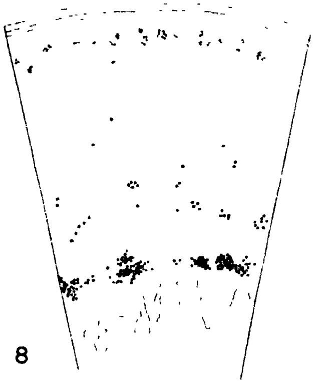

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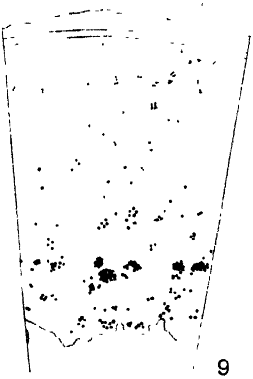

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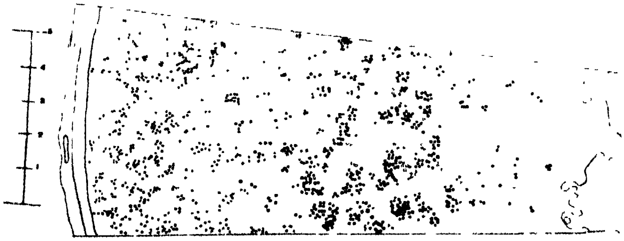

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# THE DIGITAL PADS OF THE TREE FROGS. A STUDY OF THE PHYLOGENESIS OF AN ADAPTIVE STRUCTURE

G. K. NOBLE AND M. E. JAECKLE

EIGHTEEN FIGURES

AUTHORS' ABSTRACT

The digital disks of the tree frogs are covered ventrally with a superficial layer of vertically elongated, fibrous, and distally free cells between which there empty a series of convoluted mucous glands. The latter are surrounded and, in the larger-disked species, divided into blocks by sheets of collagenous fibers. The glands are emptied by the squeezing together of the collagenous fibers when the body weight exerts a pull on the terminal phalanges. The disks function by friction, cohesion, and adhesion

The digital-disk apparatus was fully established before certain groups of frogs became arboreal. It is retained in others which have reverted to the terrestrial habit The intercalary cartilage increases the efficiency of the apparatus. It did not arise in phylogeny until after the apparatus was developed

As the digital disks vary in extent in both arboreal and terrestrial species, arborealism seems to have resulted from a chance occurrence of large disks in the smaller bodied forms, at least, there is no progressive modification of the digits toward particular habitat requirements

The subarticular tubercles of many Salientia develop typical climbing apparatus. This may or may not be correlated with an arboreal habit. In the species with the largest subarticular tubercles no apparatus is present. Arboreal salamanders exhibit no special climbing mechanism, but adhere by pressing their moist integument against the substratum.

It is known principally from the work of Schuberg ('95) and Siedlecki ('10) that the tree frogs of two very different families have evolved a tree-climbing apparatus which is remarkably similar even to histological detail. The digital tips of both are dilated into disks which are peculiar in being covered ventrally with an epidermis of fibrous, elongate cells among which empty the long-necked, convoluted glands. As neither the glands nor the distinctive epidermis were known to be present in terrestrial species, the occurrence of both on the digit tips in such unrelated types as Hyla and Polypedates seemed to afford a splendid example of identical structures apparently called forth by identical functions. Several hylids have given up their apparently original arboreal habit and live to-day, as in the case of the cricket frog (Acris), among the vegetation or rocks near the water.

Other ground dwellers have developed digital dilatations, but do not climb trees. The question arises, have these forms the same kind of pads as Hyla and Polypedates? How close is the correlation between the arboreal habit and the presence of a distinctive type of pad? Further, has such a distinctive pad developed before or after the species became arboreal? The present study of the tree-climbing apparatus of the Amphibia was undertaken with a view to answering some of these questions. To determine, if possible, whether form in this case preceded or followed function. The paper is especially pertinent at this time, for Hora ('24) has recently denied the occurrence of the specialized glands in a certain species of Hyla. No attempt has been made by previous observers to work out the phylogenesis of the pads.

## THE DIGITAL DISKS OF HYLA

The comprehensive monograph of Schuberg ('95) on the structure and function of the adhesive pads of tree frogs was based largely on Hyla arborea. This species was available to us only in a limited series, but another, much larger, and therefore more suitable species, H. dominicensis, was included in the collections of the American Museum by a large series of both living and well-preserved specimens. As the digits of the latter closely resemble in almost every detail those of H. arborea, the following description is based on specimens of H. dominicensis fixed in Zenker's solution, but other species of Hyla have been available for reference.

The epidermis covering the ventral surface of the dilatations of the digit tips of dominicensis differs from that on other parts of the body. Its thickness has been increased to six to eight layers of cylindrical or cuboidal cells, those of the outer layers being elongated in a plane perpendicular to the ventral surface. All the cells of this epidermis are striated (fig. 1). On proceeding from the inner to the outer layers, the striations gradually pass over into distinct intracellular fibers. The formation of fibers from the striations is accompanied by an increase in size of a clear space or vacuole about the nucleus of each cell. The nucleus and vacuole are gradually pushed to one side as the fibers are formed and the nucleus assumes a shriveled form. The nucleus in its extreme condition of reduction is usually densely stained by the cytoplasmic dyes (fig. 1). The intracellular fibers run parallel to the long axis of the cells. This is usually vertical to the ventral surface of the digit. The main fibers of each cell are connected with one another by many smaller cross fibers and their outer and inner ends are bound firmly together. The outer ends of the cells never flay out to form a series of brushes, even in the most worn tissues. The cells in the most superficial layer are partly separated from each other by spaces which appear wedge-shaped in transverse section (fig. 1). On a surface view of the whole pad it is found that the distal end of each cell is free from that of its neighbors. Each cell would, therefore, be able to act as a unit when pressing into the substratum.

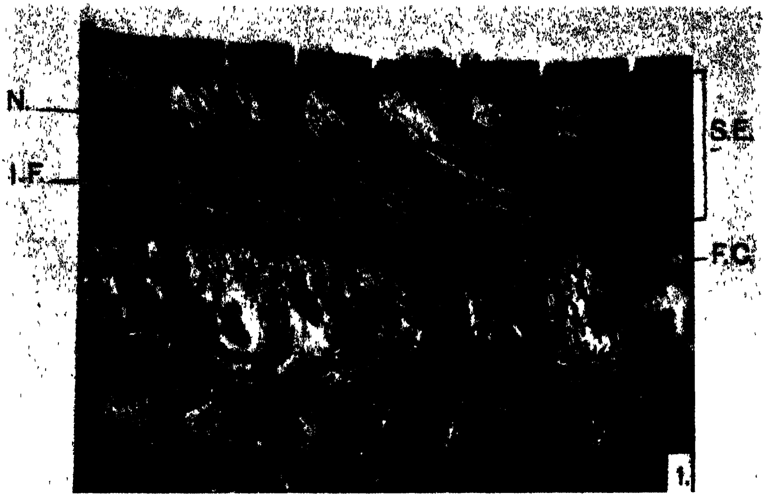
Schuberg ('95) attributes a mechanical function to the intracellular fibers. He believes they are elastic structures, 'bristles,' like the hairs of a brush, which resist shock and pressure and permit the secretion of the glands to flow into the intercellular spaces of the superficial layer. Schuberg believes that if the cells did not possess this elasticity, after the first pressure they would be permanently flattened. He admits, however, that the elasticity of the cells decreases with age, for animals which have recently shed have the outer layer almost perpendicular, while shortly before shedding the superficial cells are tilted at a considerable angle (fig. 1). Schuberg's implication that the intracellular fibers are elastic is open to objection as they do not take an elastic stain. While resorcin fuchsin, a specific elastic stain, colored the intracellular fibers a brownish purple, they could be distinguished readily from the true elastic fibers which stained a deep blue in other parts of the pad. Haematoxylin with eosin or with picrofuchsin likewise stained the fibers a brownish black. Other stains were used, and in each case the intracellular fibers retained their brownish-black color, which would indicate that they are not elastic, but some form of keratin, probably serving a protective purpose. Between the cells of the second layer, Schuberg noticed what he called short, spindle-like vacuoles, the anlage of the furrows which later partially separate the cells of the outer layer, giving them a wedge-shaped appearance. He attributed the probable origin of these vacuoles to intercellular canals. A close study of these areas has revealed that they are usually not empty, but each is filled by a flask cell similar to those described by Pfitzner ('80) and Wilder ('25).

The flask cells have a definite flask-like form; the nucleus, which is oval and fills the base of the cell, does not contain the characteristic chromatin figures. Flask cells occur where two or three epidermal cells come together, and, unlike the other epidermal cells, are not joined to their neighbors by intercellular bridges. The upper end of the cell is closely connected with the outer horny layer, the whole cell being pulled out when the horny layer is shed. The appearance of a flask cell is often so transparent that it looks like a small vacuole, but upon close observation, it is apparent that a cell actually fills the space. The protoplasm of the flask cell is somewhat lighter and more finely granulated than that of the neighboring cells. A study of the flask cells in the deeper epidermal layers suggests that they do not multiply by mitosis, but are formed from typical epidermal cells.

Observers previous to Pfitzner attributed a glandular function to these cells, but Pfitzner's interpretation that they serve a purely mechanical function is probably correct. He believes they form a close union between the horny layer and

## ABBREVIATIONS FOR ALL FIGURES

*C.F.*, collagenous fibers
*E.F.*, elastic fibers
*F.C.*, flask cells
*I.C.*, intercellular canals
*I.F.*, intracellular fibers
*I.P.*, intercalary phalanx
*M.G.*, parallel rows of mucous glands
*N.*, nucleus
*S.E.*, superficial layer of epidermis, showing wedge-shaped cells
*S.M.*, smooth-muscle strands
*S.P.*, subarticular pads
*T.P.*, terminal phalanx
*V.S.*, ventral side of disk



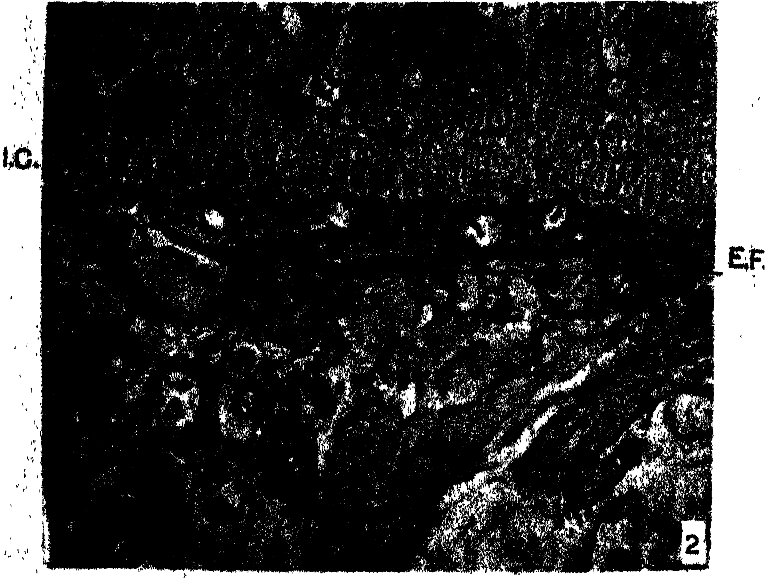


**Fig. 1** Hyla dominicensis (Tschudi). Sagittal section of epidermis on ventral surface of digital dilatations. × 1220.

**Fig. 2** Hyla dominicensis (Tschudi). Cross-section of deeper layers of epidermis and outer corium of digital disk, showing elastic fibers. × 510.

the deeper epidermis cells. Pfitzner finds they occur in all Amphibia, except the perennibranchs.

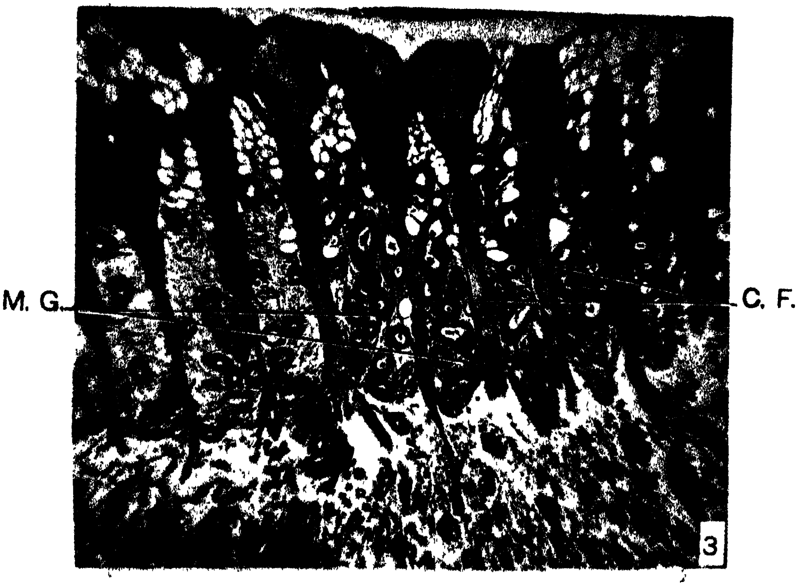
All the epidermal cells, except the flask cells, are connected by intercellular bridges. The cells of the deeper epidermal layers are more abundant, somewhat cubical in form, and more irregular in their arrangement (fig. 2). The deepest epidermal layer unites with the dermis by means of fine threads or processes from the epidermal cells which run deep into the corium, apparently to join the connective-tissue fibers. At least, they form a distinct network, thereby insuring a close union between the epidermis and the corium, which is especially necessary to prevent the epidermis from being torn off when the toe is withdrawn.

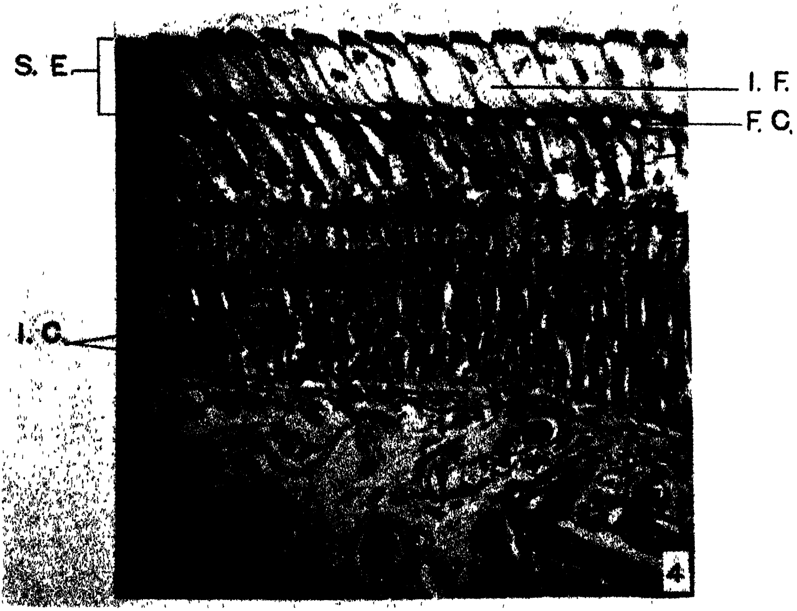
The corium is composed of a comparatively thin layer of white connective-tissue fibers interspersed with many elastic fibers which spread profusely directly under the epidermis, forming a sort of fenestrated membrane which is pierced by the processes from the epidermal cells, by connective-tissue fibers, and by glandular ducts. The elastic fibers do not penetrate into the outer layers of epidermal cells.

The deeper dermal layers are closely associated with the large glands which run in parallel rows almost the whole length of the pad (fig. 3). These glands originate in the central or upper part of the pad, continuing ventrally into shorter and narrower ducts which perforate the epidermis at an acute angle. The glands are composed of a single layer of cuboidal or slightly columnar cells with a fairly large lumen which varies in size according to the activity of the cells. From the structure of the cells and the staining properties of the secretion, as revealed by Mayer's mucicarmine and muchaematein, it is evident these are mucous glands. Numerous elastic fibers encircle the glands. Between the parallel

**Fig. 3** Hyla dominicensis (Tschudi). Frontal section of digital disk, showing parallel strands of collagenous fibers and mucous glands in deeper corium layer. × 83.

**Fig. 4** Polypedates leucomystax Tschudi. Sagittal section of epidermis of digital disk. × 423.





Figures 3 and 4

rows of glands are thick strands of collagenous fibers (fig. 3) which, like the series of glands, run the length of the pad. Some sagittal sections show only the collagenous strands, others only the series of glands. If the epidermis of the ventral surface of the pad is carefully removed, the collagenous strands can readily be seen. They are irridescent-white, closely resembling the long tendon running along the ventral side of the toe. With further dissection, these strands are seen to arise (*a*) dorsally on each side of the distal end of the next to last phalanx, not including the intercalary. Some fibers at the extreme right and left continue on the dorsal side and run toward the distal end of the last phalanx, but the majority turn toward the ventral side where some right fibers cross to the left and some left fibers to the right, to form parallel strands which continue toward the distal end of the toe (fig. 13). As the fibers wind around toward the ventral side, a few *a* fibers (fig. 14) unite laterally with the broad band of *c* fibers which covers the ventral surface of the intercalary. A small branch, *a′*, runs directly to the corium immediately lateral to the phalanx. A second set of fibers, *b*, shorter and less dense than *a*, arises directly distal to the latter. The majority of the *b* fibers have their point of insertion on the proximal dorsal surface of the last phalanx, while a smaller branch, *b′*, soon unites with *a* and follows their course. In addition to these, a third set of fibers, *c*, arises from the dorsolateral surface of the proximal end of the last phalanx. It is a broad band which runs upward over the *a* fibers, pierces them and then runs around to the ventral side of the phalanx and under the *a* fibers of the ventral surface (figs. 13 and 14), some right fibers crossing to the left and some left to the right, each fiber running into the parallel strands formed by the *a* and *b* fibers. Many of the *c* fibers are attached to the intercalary as they cross it. Thus each parallel strand which runs the long axis of the pad is composed of fibers from both the right and left components of the original *a, b,* and *c* strands of the dorsolateral surface of the phalanges. The distal ends of the parallel strands are interwoven with the short connective-tissue fibers underlying the epidermis of the pad. In this way, the phalanges as well as the intercalary are firmly connected with the corium and close coordination between them insured. Sagittal sections show the attachment of the parallel strands to the last phalanx and the capsule over the intercalary. These arrangements may be better understood by referring to the diagram (fig. 18).

The strands are not united with the tendons inserted on the dorsal (upper) side of the last phalanx. In dissecting, the tendons can be lifted off without removing the ligamentous strands. As in most ligamentous tissue, each strand is enclosed and penetrated by elastic fibers.

In his discussion of the function of the adhesive apparatus, Schuberg mentions briefly some lateral articulating bands which unite the last two phalanges with the intercalary and with each other. He did not trace to a conclusion the course of these bands and therefore failed to recognize them as the collagenous fibers after they had split into separate strands between the parallel rows of glands. While Hyla arborea possesses relatively less collagenous tissue than H. dominicensis, nevertheless, the characteristic parallel ligamentous strands are very evident in several frontal sections of a H. arborea toe which we have prepared. Schuberg believed the two large diverging bands of smooth muscle, symmetrically arranged, which run from the upper distal end of the last phalanx, and also those longitudinal strands of muscle embedded in the connective tissue between the glands were the important factors in the functioning of the adhesive pad. He does not mention the stronger and more numerous parallel collagenous strands, and apparently overlooked the part which this tissue must necessarily perform in the operation of the adhesive apparatus.

The digital disks are richly supplied with blood vessels and capillaries. No blood vessels are found within the ligamentous strands, but the glandular layers between the strands are well supplied with capillaries which follow the direction of the glands. All the capillaries in the pad are very convoluted to provide for the sudden expansion and contraction which take place.

Directly under the tip of the last phalanx at the distal end of the pad is a large lymph space which is pierced by smooth-muscle fibers and connective-tissue fibers, but which does not possess the usual endothelial boundary. There are no lymph vessels visible in our preparations of the glandular regions.

Between the last and next to last phalanx is the characteristic intercalary phalanx of the Hylidae (Noble, '25). In H. dominicensis, it is of medium size and is composed of fibrous cartilage, without a marrow cavity. The intercalary is richer in cartilage cells than the hyalin cartilage of the phalanx ends, and, upon high magnification, fibrillae can be seen in the ground-substance.

In bringing this description to a conclusion, it should be mentioned that, while the above notes were made on Hyla dominicensis, they probably apply equally well to any species of the genus with large or medium-sized disks. We have studied in serial sections the disks of the following additional species: Hyla arborea, H. baudinii, H. cinerea, H. andersonii, H. (Pseudacris) ocularis, H. (Pseudacris) triseriata, and H. annectens. The last species was the one described by Hora ('24) as lacking the pad glands. Our sections, however, reveal them as present and as fully developed as in H. cinerea. H. annectens agrees fully with the other species of the genus having medium-sized pads. The digital pads of the species of Hyla examined are not all alike. They differ chiefly in the degree of development of the ligamentous tissue between the pad glands, the species with smaller disks having less tissue, and this more irregularly distributed than in the larger species. The phylogenetic and functional significance of this difference will be discussed below.

## THE DIGITAL DISKS OF POLYPEDATES

Siedlecki ('10) based his investigations on Polypedates reinwardtii. This species of Polypedates was not available to us, but Polypedates leucomystax, which closely resembles it in most respects, was used for comparison.

The epidermis of Polypedates leucomystax is nearly identical with that of Hyla dominicensis. Siedlecki says that Hyla has smooth uniform cells along the outer surface. From the accompanying photographs (figs. 1 and 4) it is evident that the digital pads of both families have the same structure, the smoothness of the outer layer depending upon the time before shedding. Siedlecki believes that cells and fibers from the corium run in between the epidermal cells, bringing not only the deeper layers, but all the epidermal cells except those of the two outer layers into direct contact with the connective-tissue elements of the corium. In leucomystax we found connective-tissue fibers only in the deepest layers of the epidermis where the epidermal processes formed a network with them. On a slide stained with picrofuchsin, where the epidermis stains yellow and white fibers pink, there is no trace of pink fibers between the yellow cells of the outer epidermal layers. Likewise, on a slide stained with Mallory, no connective-tissue fibers which would stain blue were seen between the red epidermal cells. However, there are intercellular canals and bridges which extend from the outer layers to the corium, and what Siedlecki described as white fibers in his photographs are probably the canal-like intercellular spaces between the epidermal cells, as he makes no mention of intercellular bridges or canals. Siedlecki does not mention the flask cells, which are very obvious in Polypedates leucomystax, as well as in P. reinwardtii (Siedlecki, '10, fig. 5).

The glands in Polypedates and Hyla are similar in structure and apparently in function. Siedlecki, however, describes these glands as serous, with a few mucous cells in each gland. After several staining tests (mucicarmine and muchaematein) for mucus and after a comparison with the known mucous skin glands in the same preparation, it is evident that these are entirely mucous glands, and what Siedlecki describes as a few mucous cells in each serous gland are the active mucous cells filled with the flocculent secretion. The other cells are at rest following a discharge of their secretion, which can be seen in the lumen of the gland.

There is relatively less collagenous tissue in proportion to the glands in Polypedates than in Hyla, where the ligaments are greatly developed. Except for this difference, the arrangement of pad elements is nearly identical in the two genera. Siedlecki gives an accurate description of this tissue so far as the proximal part of the pad is concerned, but he believed the distal pad was composed of thick strands of smooth muscle, running parallel to the long axis of the pad, and reinforced with connective-tissue and elastic fibers. However, what Siedlecki terms the muscular lamellae are actually continuations of the parallel collagenous fibers described by him in the proximal end of the pad, and not smooth muscle. Histologically and structurally, these fibers meet the requirements for collagenous tissue. The accompanying chart shows the different staining reactions which were obtained between smooth muscle and the parallel collagenous fibers not only in Polypedates, but also in Hyla dominicensis and H. arborea. When highly magnified, the individual muscle cells, with their characteristic nuclei more or less centrally located, can be distinguished from the collagenous fibers and cells which resemble tendons in their composition.

| STAIN | PARALLEL COLLAGENOUS FIBERS | SMOOTH MUSCLE | WHITE FIBERS | ELASTIC FIBERS |
|---|---|---|---|---|
| Picro-fuchsin | Pink or red | Yellow-orange | Pink or red | Light yellow |
| Mallory | Blue | Red | Blue | Yellow or pink or blue |
| Eosin | Light pink | Dark pink | Light pink | |

Siedlecki gives a detailed description of the blood vessels and capillaries in the pad of Polypedates. This agrees with the conditions in Hyla. The large lymph space on the dorsal side of the distal part of the pad is found in both genera, but, contrary to Siedlecki's investigations, no lymph vessels were found within the ligamentous strands of the central part of pad.

The intercalary of Polypedates is large and wedge-shaped. It occurs only in adult animals as fully ossified bone with a marrow cavity and cartilaginous articulating surface. It differs in this respect from Hyla, where the intercalary is composed of fibrous cartilage. In younger animals the intercalary is still composed of hyalin cartilage as in some species of Hyla.

The terminal phalanges of Polypedates are T-shaped, in Hyla claw-shaped. Other differences appear in the outer form of the pad. In Polypedates a groove marks off a small disk on the distal ventral part of each terminal dilation. This groove is not specialized histologically. The peculiar superficial layer of fibrous epidermis is found both proximal and distal to it. Thus, the disk-like formation on the ventral surface of the digital dilatation of Polypedates does not mark off a functional pad from an indifferent integument as one might be led to conclude from a superficial examination of the pads. The lateral and distal parts of the groove, however, lying practically on the edge of the dilatation, limit more or less the distribution of the specialized, fibrous epidermis in these directions. Pronounced grooves are not found on the dilatations of Hyla. The digital disks of Hyla and Polypedates differ somewhat in external appearance and in the form of the terminal phalanges, but they are essentially alike in their histological structure.

## THE PHYLOGENESIS OF THE ADHESIVE APPARATUS

How did tree frogs of two very different families happen to develop a digital adhesive apparatus so similar in histological detail? In endeavoring to answer this question we have studied the structure of the digits of a great many genera and species of Amphibia.

In most terrestrial and fossorial Salientia of all families the integument covering the digit tips is slightly thickened, but does not possess a fibrous epidermis. The epidermal cells agree essentially with those on the dorsal surface of the hand. No elongated and convoluted glands are found emptying on the tips of the digits, but ordinary mucous and rarely granular glands, similar to those on other parts of the integument, are found in these regions. This is the condition in such taxonomically widely separated species as Ascaphus truei, Alytes obstetricans, Scaphiopus holbrookii, Atelopus varius, and Rana pipiens.

In some species of different families the digit tips are slightly dilated to their tips. These frogs are not arboreal and they are usually described as not possessing pads. We have examined a few of these species. In the common wood frog, Rana sylvatica, the digit tip is dilated and frontal sections of the digits (fig. 5) reveal that certain mucous glands of the ventral surface have developed very long necks, their bodies or secretory portions coming to lie in the central part of the digital dilation. A comparison of such glands with those in the pad of the metamorphosing Hyla andersonii has shown a great similarity. In both it is a mucous gland which has developed a long neck and begun to invade the deeper tissues of the pad. In Rana sylvatica, however, the superficial epidermal cells, although slightly free one from the other, are not elongated to form a typical fibrous layer. The epidermis covering the joint swellings, the so-called subarticular tubercles, has the same appearance as the epidermis on the ventral surface of the digit tip in Rana sylvatica.

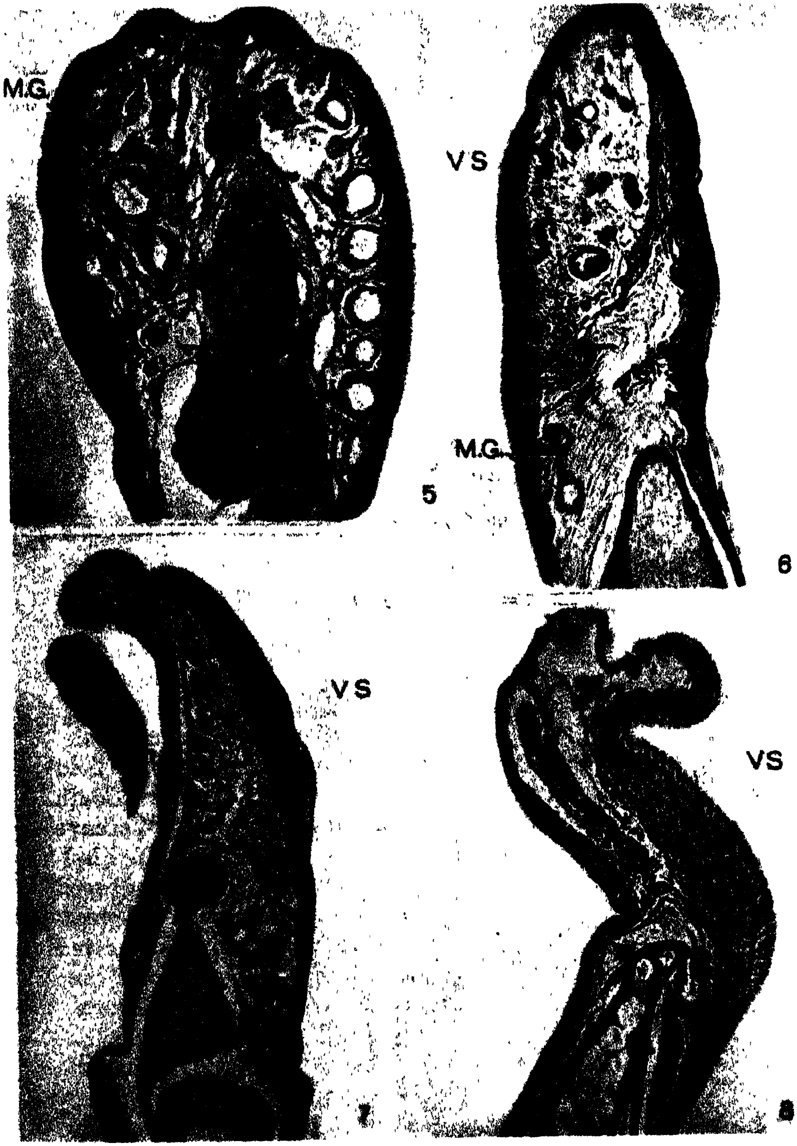
In the Panama Eupemphix pustulosus the digit tips are very slightly swollen, but there is no suggestion of pads visible externally. Sagittal sections of the digit tips, how-

Fig. 5 Rana sylvatica Le Conte. Sagittal section of digital disk. × 96.

Fig. 6 Rana warschewitschii (Schmidt). Sagittal section of digital disk. × 41.

Fig. 7 Phyllobates latinasus (Cope). Sagittal section of digital disk. × 174.

Fig. 8 Phrynobatrachus dendrobates (Boulenger). Sagittal section of digital disk. × 78.



Figures 5 to 8

ever, show some of the mucous glands are elongated as in Rana sylvatica. The epidermis is unspecialized. Obviously, the mucous glands in this species have begun to hypertrophy before there was any outward indication of a pad.

In the two Central American frogs, Rana palmipes and R. warschewitschii, the digit tips are slightly broadened, in the last species more than in R. sylvatica. Sagittal sections of digit tips of these species (fig. 6) show that the superficial layer of epidermis, although low, is distinctly of the Hyla type. The mucous glands are more elongate than in Rana sylvatica. So far as depth of the superficial epidermal layer and glands is concerned, these species afford a fair intermediate between the conditions in R. sylvatica and a typical Hyla. Nevertheless, it is clear from the observations of Halter in Nicaragua (Noble, '18) that both of these species are terrestrial or, at least, frequent either wood or stream. Another terrestrial frog, but one of a different family, having an incipient adhesive apparatus, is the Chinese Microhyla pulchra. None of these frogs is known to climb trees, and yet in all the fibrous epidermis, the elongated glands, and the ligamentous strands have already differentiated as distinctive structures.

In dealing with foreign Salientia there is always an element of uncertainty as to the habits of the species, for field observations on them are usually not extensive. A few South American frogs have very small but distinct disks. We have selected two which are very probably terrestrial. In Phyllobates latinasus (fig. 7) and Elosia bufonia a well-developed fibrous epidermis and a mass of convoluted glands are present in each digit tip. The glandular mass is not separated into sections by sheets of ligamentous tissue, but a fibrous capsule surrounds the mass which lies mostly under the terminal phalanx. A comparison between this apparatus and that in certain small hylids known to be tree frogs, such as Centrolenella parabambae, will reveal no marked differences. The parallel sheets of ligamentous tissue found in Hyla and Polypedates are reduced in small species of these genera or related forms. However, the essential adhesive mechanism of tree frogs, the fibrous epidermis, the convoluted glands, and a ligamentous capsule for the glands is present in Phyllobates and Elosia as well as in Centrolenella.

There are a number of other small frogs with digit tips more or less dilated. Some of these species are known to be terrestrial and none is known to be arboreal. We have studied the South American Dendrobates tinctorius and the African Phrynobatrachus dendrobates (fig. 8) and have found their digit tips when sectioned to agree essentially with Phyllobates latinasus. It is certain that some, if not all, of these species are primarily, if not entirely, terrestrial forms, and yet they have a fully developed adhesive apparatus. The conclusion seems obvious that the tree-climbing apparatus developed before the frogs began to climb trees.

The typical tree frogs Hylidae and the Old-World tree frogs Polypedatidae are both characterized by the possession of an intercalated cartilage or bone—an extra phalanx—between the two terminal phalanges. Most of the genera in these families are tree frogs, and it would seem that the intercalated cartilage was an essential part of the adhesive apparatus. A number of ranids, brevicipitids, and bufonids, however, have broad terminal dilatations which they are known to use in climbing trees. Is the presence or absence of this extra phalanx correlated with marked differences in the structure of the apparatus? We have studied in sections Rana (Staurois) ricketti, Platyhyla grandis, and a series of species of Eleutherodactylus. In all of these the apparatus was essentially like that of large species of Hyla and of Polypedates. In Platyhyla the parallel ligamentous septa were broad and not so compact as in Eleutherodactylus or Hyla. All the species had the same type of epidermis, glands, and ligamentous tissues. Thus, it seems certain that in hylids which sprung from bufonids (as Eleutherodactylus) and in polypedatids which arose from ranids (as R. ricketti) the adhesive apparatus was already fully established before the intercalary cartilage was added to it as a final specialization.

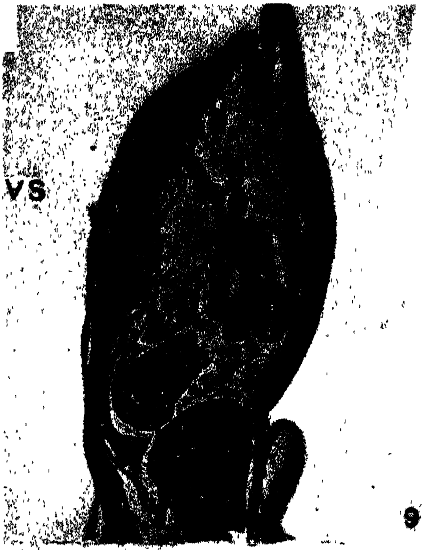
That this is the correct interpretation seems certain from the conditions in a little Central American frog which one of us described some time ago as Hyla chica. In this form the terminal phalanges of the fingers are either slightly T-shaped, knobbed, or typically claw-shaped, but those of the toes are claw-shaped. Nevertheless, the terminal phalanges of all the digits slide back under the ends of the penultimate phalanges as in Hyla. Intercalary cartilages, however, are not present (fig. 9) and therefore, according to the most recent definition of the family, the species could not be a Hyla or a hylid. An examination of the collections of Salientia in the British Museum has revealed that Boulenger had previously described the same form under the name of Hylodes (= Eleutherodactylus) gularis. No other species of Eleutherodactylus has claw-shaped phalanges and in none do the terminal phalanges slide under the penultimate phalanges Hyla fashion. The species in question appears, so far as its diagnostic features are concerned, to be an exact intermediate between Hyla and Eleutherodactylus. When, however, we study its entire anatomy, other characters appear, such as the large prefrontals in broad contact, the vomerine teeth (when present) far behind the choanae, the large unpigmented eggs (2 mm. in diameter in females, 15 to 17 mm. head and body length) which make it appear certain that the species is an Eleutherodactylus which has advanced toward the Hyla condition both in the loss of the arms to its T-shaped phalanges of the toes and in the overlapping joint between the last two phalanges of all the digits. All that would be necessary to make a Hyla out of gularis would be to further reduce the cross-pieces of terminal phalanges on the fingers and to

Fig. 9 Eleutherodactylus gularis (Boulenger). Sagittal section of digital disk. × 183.

Fig. 10 Acris gryllus (Le Conte). Sagittal section of digital disk. × 178.

Fig. 11 Eleutherodactylus schmidti Noble. Sagittal section of digital disk. × 72.

Fig. 12 Nectophryne afra Buchholz and Peters. Sagittal section of digital disk. × 82.



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Figures 9 to 12

add cartilaginous menisci between the last two phalanges at the point where they rub past each other. It is by no means certain that gularis is the actual ancestor of the genus Hyla, but its adhesive apparatus bridges the gap between that of the Bufonidae and that of the Hylidae.

We have examined the digital pads in a number of Hylidae and have found all disks of one size to be essentially alike in histological structure. The slight differences in those of Hyla dominicensis, H. baudinii, H. andersonii and Cerathyla johnsoni would seem to be chiefly associated with the difference in size. In Phyllomedusa hypochondrialis the intercalary cartilage is thin and the fibrous epithelium is less developed than in the other species of Hylidae examined. There are a number of Hylidae which have given up arboreal life entirely. The digital dilatations in these forms are small. Perhaps the best example is the cricket frog, Acris gryllus (fig. 10), but the swamp tree frogs Hyla (Pseudacris) triseriata and ocularis hide in wet meadows and probably never climb trees. We have cut sagittal sections of the digits of these species and have found in all the fibrous epidermis, the tubular glands and the ligamentous tissue of Hyla. The glands for the most part are not divided by ligamentous bands, but, as stated above, this lack is correlated with their small size. The ligamentous bands have about the same distribution as in the arboreal Centrolenella. It is therefore clear that these species which merely hop about among water lilies, wet rocks, and submerged grass stems have the same tree-climbing apparatus of their most arboreal relatives. What prevents them from leaving their lowly home and seeking a shelter among the tree tops? The question was nearly the same in the terrestrial Kassina senegalensis, which is said to lack digital disks entirely, but actually has a great mass of glands, even though the fibrous epidermis is not as enlarged as in the related but arboreal Leptopelis rufus.

We have studied this question most closely in the species of Eleutherodactylus from Santo Domingo, for one of us has made a detailed study of their habits in the field. Eleutherodactylus inoptatus is a large species which usually hides itself during the day high up in forest trees. E. schmidti (fig. 11) and E. weinlandi are smaller species which inhabit the forest floor, but are abundant only near streams. Sections of the pads of these three species reveal no important differences, and yet the first is arboreal and the two latter terrestrial species. An external examination of the disks of all the species of the genus in Santo Domingo shows that only those species climb which have large disks in proportion to their body size. Thus, arborealism seems merely a matter of disk size versus body weight. Any species which by a chance mutation develops larger disks than its fellow frogs is immediately equipped to climb, for the tree-climbing apparatus is already present in the terrestrial forms.

Conversely, any arboreal species which by chance develops smaller disks would necessarily be forced to gain a livelihood on the ground. But such a frog would not lose its tree-climbing mechanism. All species of the genus retain the apparatus whether they are purely arboreal, terrestrial, or semifossorial. Only one species in Santo Domingo, E. ruthae, can be described as semifossorial, and in this form the fibrous epidermis is not well developed. It is therefore possible that a fossorial life may tend to destroy the distinctive epidermis of the adhesive pad. Further, forms which live on damp leaves or rocks might be expected to use their adhesive pads in assuring a grip before leaping, while desert species or digging forms would have little use for their adhesive apparatus.

Nevertheless, the African brevicipitid, Phrynomerus bifasciatus, which seems to be a typical burrowing toad, possesses convoluted glands, an intercalary cartilage, and a low fibrous epithelium. The tree-climbing apparatus can therefore occur even in fossorial Salientia.

Arborealism in the Salientia is not, however, merely a matter of size—weight relationship between disk and body. There must be physiological differences which control a species activities. The Santo Domingan species of Eleutherodactylus are well segregated in different habitats. The little E. abbotti can climb very well, but seldom ascends more than a few feet from the ground. On the other hand, E. auriculatoides was never found near the ground, although it is also a small, fairly smooth-skinned frog. E. flavescens occupied a habitat on the tops of bushes at an intermediate height between the two extremes. We are entirely ignorant of the causes of this habitat preference, but it is probably associated with different humidity levels rather than with any difference in the tree-climbing apparatus.

The lack of correlation between the histological structure of the pad and the habitat of the species is further exemplified by an extension of the pad which covers the subarticular swellings. These enlargements may be covered with the same distinctive epidermis found on the terminal dilatations, and in a few cases may be provided with convoluted glands. In Eleutherodactylus inoptatus, E. schmidti, and E. weinlandi the distal subarticular tubercles are covered with this specialized epidermis. The terrestrial Acris gryllis has both modified epidermis and glands on the subarticular tubercles, while in most arboreal Hylas the epidermis is not greatly modified over the joint swellings. On the other hand, subarticular pads with modified epidermis are found in Polypedates leucomystax, Phyllobates latinasus, and Elosia bufonia. It is clear that the extension of the pad epidermis is not correlated with an arboreal habit.

A few Salientia have developed enormous subarticular swellings or pads. We naturally expected that these would show a great hypertrophy of the epidermis and glands. Such was not the case. In the bufonid, Nectophryne afra (figs. 12 and 16) and the brevicipitid Phrynella pulchra (fig. 15) the subarticular pads, although of great extent, are entirely devoid of fibrous epidermis. Further, only very few or no mucous glands are elongated in these pads. Thus, the mechanism of adhesion must be totally different in these forms than in most tree frogs.

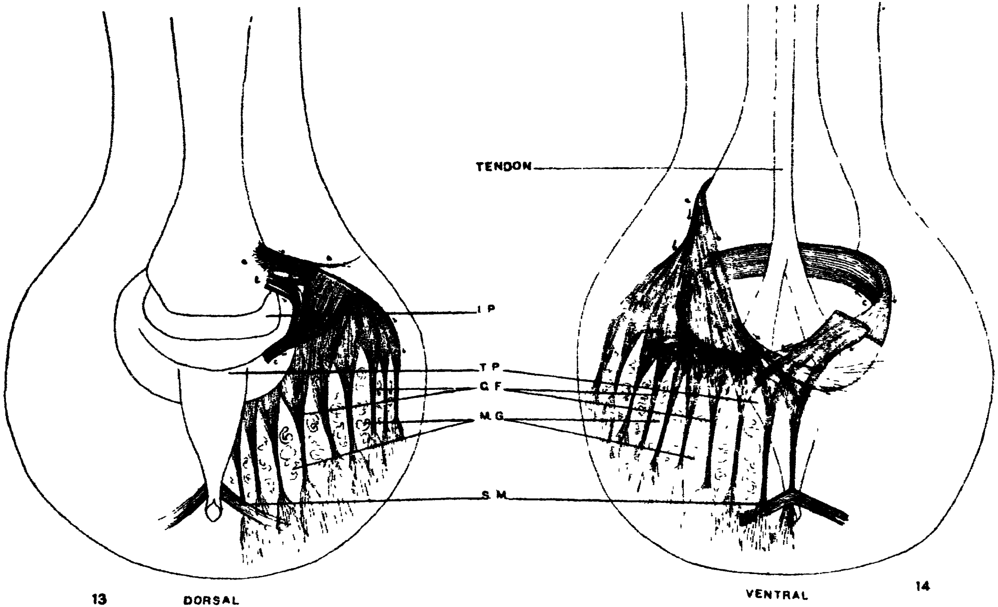
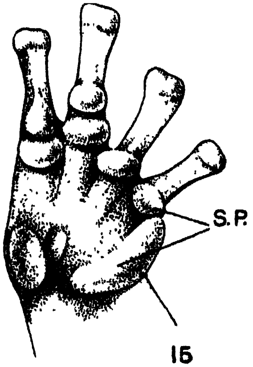


Fig. 13 Diagrammatic dorsal view, showing origin and course of collagenous fibers in Hyla dominicensis (Tschudi) digital disk.

Fig. 14 Diagrammatic ventral view, showing path of collagenous fibers in Hyla dominicensis (Tschudi) digital disk.

The mechanism in these forms is apparently the same as in arboreal salamanders. We have studied sections of the broadly webbed toes of Hydromantes italicus and have found the epidermis smooth and non-fibrous as in Nectophryne. Further, no enlarged glands are present. Of the various local salamanders which we have kept in terraria, Plethodon glutinosus can climb most easily up the glass walls. On various occasions we have found individuals of this species adhering to the top of the aquarium just under the glass cover. Sections of the digits of Plethodon glutinosus reveal no specialized epidermis or enlarged glands. Plethodon glutinosus is not arboreal in nature, although it is obviously able to climb trees.



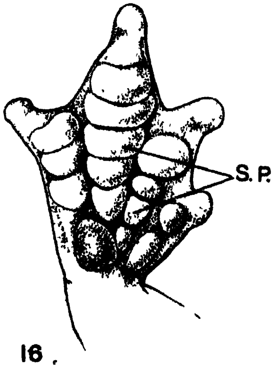


Fig. 15 Phrynella pulchra Boulenger. Right manus. Ventral view.
Fig. 16 Nectophryne afra Buchh. and Peters. Right manus. Ventral view.

The inverse ratio between climbing ability and body weight is well seen in the species of Desmognathus. We have kept four species of different sizes in similar glass-covered tanks for some weeks. The large D. quadra-maculatus during this period (although able to do so, as was later proved) was never found to climb the sides of the tanks. The slightly smaller D. phoca was frequently found on the stone ends to the tanks, but not on the glass faces. The still smaller D. fuscus was frequently seen on both glass and stone walls. The smallest form, D. f. carolinensis, was often found creeping upside down across the moist glass cover to the tank. In none of these species is any special mechanism developed for climbing. The smaller the species, the better fitted it is to adhere by cohesion and adhesion to moist surfaces.

On the other hand, at least one terrestrial salamander has developed elongated mucous glands on the tips of its digits. As pointed out long ago by Leydig ('76, pl. XI, fig. 136), Salamandra atra has a series of elongated mucous glands at the digit tips, but these glands are not convoluted as in the tree frogs and they lack a pronounced lumen. Just how they function is unknown. Leydig (op. cit.) errs, however, in describing the dense connective tissue between the last two phalanges as an intercalary cartilage. No definitive cartilage cells are present in this connective tissue and the latter is firmly bound to the phalanges. Similar syndesmotic joints are found in Alytes and Ascaphus.

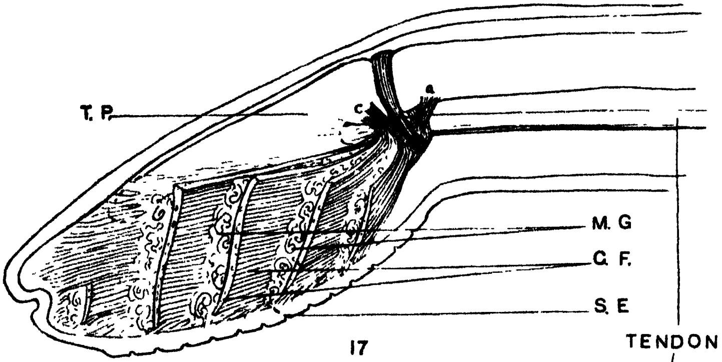
In the salamanders, therefore, certain slim-bodied species are able to climb smooth vertical walls and a few species with webs between their toes are normally arboreal. No modification of epidermis or mucous glands assist these species in their efforts, although elongated mucous glands occur at the digit tips of one purely terrestrial species.

## THE FUNCTION OF THE ADHESIVE APPARATUS

It is clear from the preceding sketch that the adhesive apparatus of the digit tips of Amphibia arose before the animals became arboreal and, further, has been retained in many, if not in all, species which have reverted to the terrestrial habit. Thus, the functional significance of the apparatus as a whole would seem to be to insure a firmer grip on the substratum whether the latter be in a vertical or a horizontal plane.

The different parts of the apparatus play varying rôles in the process of adhering. It would seem obvious that the superficial layer of fibrous epidermis would function in increasing the friction and that the mucus from the convoluted glands would function either to increase the adhesion or the cohesion of the pad against the substratum. The function of the intercalary cartilage, when present, is not so clear, and our interpretation of the relative importance of the different parts of the mechanism is at variance with that of previous writers. Schuberg ('95) attaches considerable importance to the intercalary cartilage and the smooth muscles in the movements of the terminal phalanx and the flow of mucus. The smooth muscles in the distal end of the pad may be instrumental to some extent in causing the glandular secretion to exude, but Schuberg overlooked the much more important part which the parallel strands of ligamentous tissue between the glands must necessarily play. The weight of the frog pulling the end phalanges away from the adhering pads would cause the fibers to stretch and approximate each other. These strands would squeeze the glands in the same way that a cluster of parallel ropes, if brought together at one end and pulled, would squeeze any object inserted between them. In Hyla, Polypedates, and all tree frogs with large pads the glands are emptied primarily by the pressure of the ligaments, not by the smooth muscles, which are much fewer than either Schuberg or Siedlecki surmised. As stated above, the parallel strands of tissue between the glands, which both Schuberg and Siedlecki considered smooth muscle, we have shown to be ligamentous tissue. In species with small pads, whether arboreal or terrestrial, the glands are not separated by strands of ligamentous tissue. However, the glands are usually surrounded by a capsule of dense connective tissue which adheres to the terminal phalanx. A pull on the phalanx would, even in these cases, be transmitted to the gland. In short, it seems that the rôle of smooth muscle in emptying the glands is very little, if any. On the other hand, a very simple mechanism is present in all species with pads to transmit the pull exerted on the terminal phalanges into a compression action on the glands. The smooth muscles present may play a part in controlling the form of the pad or even in exerting a slight pressure on some of the glands. The main pressure, however, is exerted through the pad ligaments.

The intercalary bone, when present, seems to increase the efficiency of the apparatus. As shown in the accompanying diagram (figs. 17 and 18), the intercalary permits a greater amount of adhesive surface supported by bone. Hylas slipping off the side of a smooth rock will be seen to raise and lower the tips of their terminal phalanges. In this act that part of the adhesive apparatus at the base of the terminal phalanx remains closely pressed to the rock, while the distal part makes a series of attempts to secure a better hold. In forms without the intercalary no part of the pads would re-



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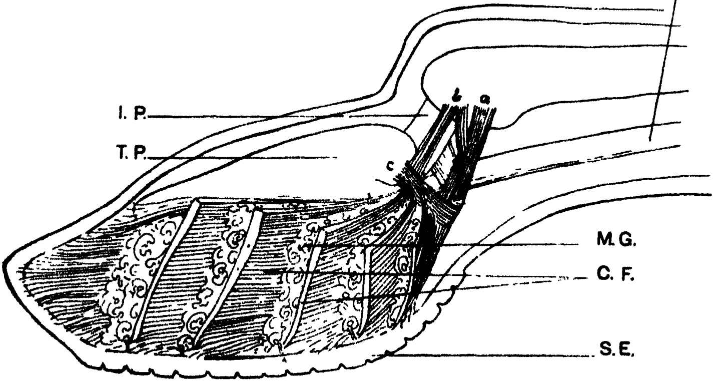

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Fig. 17 Schematic sagittal section of digital disk of Eleutherodactylus.
Fig. 18 Schematic sagittal section of digital disk of Hyla.

main tightly in contact with the surface if the tips of the terminal phalanges were raised. This may be better recognized by comparing the diagrams. That the intercalary cartilage is not essential to arboreal life is attested to by the great numbers of arboreal frogs which have evolved an adhesive apparatus identical to that of Hyla, but lacking the intercalary. A comparison of the climbing abilities of the species of Eleutherodactylus with the Hylas in any given region will yield little in favor of the latter. Further, there are many groups of frogs having the intercalary which are purely terrestrial in habit. Most of these have reverted to terrestrial life. At least one, however, Phrynomerus, may never have been arboreal. The intercalary has usually arisen in connection with arborealism, but possibly not always.

The function of the fibrous epidermis would seem to be to increase the friction. Schuberg ('95), however, believes that the interspaces between the superficial cells may permit the mucus to flow more freely. This may be true, but it seems hardly likely when similar epidermis is found in some species over the articular tubercles which usually lack glands. The fibrous epidermis may be compared directly with the 'sucker' on the gecko's foot. The comparison is fairly exact in the case of Sphaerodactylus, where there is found a single undivided patch of bristles on the dilatation at the end of each digit. These minute friction pads, although devoid of glands, support the weight of the little lizard so well that it can run upside down across the ceiling. There have been various theories advanced as to the method of functioning of the digital dilations of geckos (Hora, '24), but it seems that in Sphaerodactylus the pads function by friction alone, for in this case there is merely a single pad of minute bristles at the end of each digit. No Amphibia climb by the aid of friction pads alone, but in no species is the bristle tissue as highly developed as in these geckonid lizards.

Siedlecki ('10), in discussing the function of the pads, points out that specimens of Polypedates, which have been so badly injured that both glands and the distinctive epidermal layers are destroyed, can, nevertheless, adhere. He concludes (p. 606, translation): "It is therefore very apparent that the function of the apparatus cannot rest upon the stickiness of the glandular secretion." This discouraging conclusion is certainly not well founded. Most tree frogs use their abdomens and appendages to supplement the action of the pads. But if certain individuals are able to adhere by the accessory apparatus alone, it does not follow that the primary apparatus does not normally function. We experimented on Hyla septentrionalis, a moderate-sized tree frog with well-developed pads, by removing the pads on all the fingers and toes while the animal was under ether. The next day the injured frog and a normal one of the same species were placed in a tall glass jar. When held at an angle of 90° the injured Hyla slipped down along the side of the jar as the pads of the fingers and toes did not take hold. The accessory apparatus of the abdomen and appendages was sufficient, however, to prevent the animal from falling off. The normal frog retained a firm hold on the sides of the glass with its toes and fingers and did not slip at all. At an angle of approximately 100°, the injured frog was unable to stick and fell off immediately, while the normal one slipped slowly down the side of the jar, but did not lose its hold. When the jar was turned upside down, both animals fell off, but the normal frog was able to remain a few seconds longer than the one without pads. Further evidence of the adhesiveness of the terminal pads is given by turning one's hand upside down after a tree frog has secured a firm hold to the upper surface. It is apparent that the sticky secretion of the ventral surface of the body and the appendages is not sufficient to support a tree frog of moderate weight.

## THE DIGITAL DISKS AS ADAPTIVE STRUCTURES

It has been universally conceded that the digital disks of the tree frogs are highly adaptive structures. From the data presented above, it seems clear that these structures arose not through a progressive change directed toward the elaboration of a climbing mechanism, but merely by an accidental association of the species with arboreal life after a modification of the digits had developed. Such a method of adaptation is in keeping with the known facts of genetic research, but is at variance with the views vigorously defended by many biologists who do not believe in the all-sufficiency of these variations. Guyer ('25) has recently expressed himself: "It seems more within the bounds of credence to suppose that biologists have failed as yet to discover some more direct principle of adaptation whereby living organisms are fitted to their environment." Probably most field naturalists hold the same opinion.

A detailed analysis of the many 'marvelous adaptations' in the Amphibia will reveal, we believe, that in most cases the modification arose before the function. A bird's-eye view of the order Salientia as a whole will give the impression that the genera have arisen merely by a shuffling about of characters. The form of the pupil, the loss of teeth, the development of tooth-like structures in the lower jaw have apparently no correlation with specific habitats (Noble, '22). Parallel changes in unrelated groups have occurred many times. Mention may be made of the hooked terminal phalanges of Rana christyi and Gampsosteonyx or the loss of the clavicle in unrelated brevicipitid stocks. It is difficult to assign any function to these and many other skeletal changes in the group.

One skeletal change, which has frequently been cited as adaptive, is the loss of teeth in the Amphibia. Among urodeles various plethodontids may lack either the vomerine or maxillary teeth in the male. The functional significance of this loss is unknown. Among the Salientia the genera of different families have independently lost their teeth entirely. Frequently, the modified genus and its immediate ancestor live side by side. Familiar examples are found in Phyllobates and Dendrobates, Paludicola and Eupemphix, Crinia and Pseudophryne, Arthroleptis and Cardioglossa, etc. The stomach contents of one of these pairs of genera have been examined, and no difference in the food habits has been observed (Noble, '24). With very few exceptions, Salientia seize any moving object within their reach. Frogs and toads are capable of distinguishing by experience the obnoxious objects from the edible ones (Schaeffer, '11). Further, certain burrowing forms gorge themselves on the termites, but whether through choice or merely because these are the dominant insects in their habitat is unknown. In general, very few, if any, Salientia seek out specific foods upon which they feed. The bulk of the evidence seems to point definitely to the conclusion that the teeth have been lost without any reference to food habits of the animal. In some cases we may say that the absence of teeth is correlated with a termite diet, but in many more cases there is no such correlation.

Adaptations of this inexact type are frequent among the Salientia, and in all cases it appears that the modification developed before it was found to be of any use. Our description of digital disks of Hyla were based upon dominicensis, and in selecting an example of an inexact adaptation reference may be further made to this species. Dunn ('26) has recently shown that the closely related form H. lichenata lives in tree cavities. The secondary deposit of bone which covers the skull of this species as a grotesque casque would seem to function in plugging the hole in which the creature passes the day. But large specimens of dominicensis, especially the females, develop a similar although less sculptured bony cover to the skull. A large series of this species has been collected, but the habit of using the head as a plug has never been observed in dominicensis. Many species of Hyla withdraw in holes in trees, as, for example, the common versicolor, and yet the casque formation is of rare occurrence in the genus. Naturalists have always been eager to seize upon any peculiar modification and assume that it must have some particular function. A closer study usually reveals that these structures are much less adapted for particular ends than originally assumed.

The Salientia have possessed their present body form and principal skeletal features for about seventy million years, that is, since the Jurassic. During this tremendous time they seem to have made little advance in the development of radically different types. Nevertheless, some groups, such as Eleutherodactylus and Hyperolius, are evolving rapidly, as shown by the enormous number of species in limited areas. It has frequently been stated that most specific characters do not have a survival value, and this would seem to hold true for the Salientia. Take, for example, the kaleidoscopic color differences in the species of Hyperolius or the different web lengths in Hyla. The hereditary mechanism of these forms would seem to be turning out numerous variations, some of which are obviously useful, but most of which seem to have no survival value to the species.

As regards the tree-climbing apparatus of the Amphibia, it seems clear from the above data that this developed without any relation to the tree-climbing habit. Slim-bodied salamanders climb without any mechanism other than their normally adhesive integument. The specialized pad structures of the tree frogs (epidermis, glands, and ligamentous tissue) are well developed in species which are terrestrial and have no arboreal ancestors. The Salientia with the most extensive pads are believed to be arboreal, but here the habit data is incomplete. The pad apparatus is retained fully developed in terrestrial forms which have arisen from arboreal ancestors. Variations in pad extent would seem to be the primary factor in determining whether a species will be arboreal or terrestrial, but there are many secondary factors, as stated above, which may limit the actual migrations of a form.

## CONCLUSIONS

1. The digital disk apparatus of the Amphibia consists of a superficial layer of fibrous and partly separated cells among which there empty a series of convoluted mucous glands. The latter are either surrounded by a capsule or are divided up into a series of blocks by a series of compact strands of collagenous fibers. In the larger disks the strands are parallel and run the long axis of the disk.

2. In functioning, the superficial epidermal layer grips the substratum by friction. The body weight of the frog exerts a pull on the distal phalanges which is transmitted to the collagenous fibers of the disk. The glands are emptied automatically by the squeezing of the ligamentous strands, and not by the action of smooth muscle, as previous writers have described. The secretion of the glands when applied to the surface of the disk functions both through adhesion and cohesion.

3. The intercalary cartilage or bone increases the efficiency of the pad mechanism, but all the other components of the pad mechanism were established long before the intercalary was formed in phylogeny.

4. Arboreal urodeles lack all trace of the digital disk apparatus, although one terrestrial species has tubular glands on the digit tips. They are able to climb by pressing a large area of normal integument against the substratum.

5. A few arboreal frogs (Nectophryne afra, Phrynella pulchra, etc.) have developed large subarticular pads on the digits, but small terminal disks. These subarticular pads lack the fibrous epidermis and have very few or no glands. This is remarkable, for in other tree frogs with terminal disks the subarticular tubercles may have a typical pad structure.

6. The digital disk apparatus was fully established before certain groups of frogs became arboreal. It is also retained in others which have returned to the ground.

7. Ability to climb is primarily dependent on a relationship between disk area and body weight.

8. As the digital disks vary greatly in both arboreal and terrestrial species without any obvious correlation with specific environments, it would seem that arborealism in the Amphibia was merely a chance relationship between large disks (or adhesive areas) and a relatively light body weight.

9. Many other so-called highly adaptive structures in the Amphibia are as fortuitous in their occurrence as the large digital disks. Adaptation in these cases is a chance relationship, not a progressive modification toward particular habitat requirements.

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# SPIRAL MOVEMENT IN MAN

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TEN FIGURES

AUTHOR'S ABSTRACT

Blindfolded persons walk, run, swim, row, and drive automobiles in clock-spring spiral paths of greater or less regularity when attempting a straightaway. The spirals turn either right or left in one and the same individual, and may do so even in one experiment. But either right or left turns predominate in the great majority of individuals, often to a high degree. The paths show marked individuality, and there is some ground for thinking there exists a correlation between temperamental differences and general character of path.

The mechanism which produces the spiral path is not located in the locomotor organs, but in the central nervous system and is probably identical essentially with the spiral mechanism in other motile organisms, all of which move in spiral paths when there are no guiding senses to direct the path. The clock-spring spiral in man is interpreted as the expression in two dimensions of space of a helical spiral mechanism which seems to exist in all motile organisms moving in three dimensions of space and in amebas which move in two dimensions. In a large number of lower organisms the number of body lengths per spiral turn is almost constant, being about 4.5. The smallest regular swimming spirals in man are very close to this value, but the smallest regular walking spirals are somewhat larger. The fundamental spiral mechanism seems to be of molecular dimensions, and there seems to exist a demonstrable locomotor bilateral asymmetry in very nearly, if not quite, all organisms.

## CONTENTS

Introduction .......... 294
  Historical .......... 299
  Methods of work .......... 302
Experimental observations .......... 306
  Walking straightaway blindfolded .......... 307
  Driving an automobile blindfolded .......... 314
  Walking around imagined circles .......... 318
  Swimming straightaway blindfolded .......... 324
  Effect of a drug on spiral movement .......... 333
  Spiral movement in two mental defectives .......... 335
  Walking around figure-8's and around squares .......... 338
  Spiral movements on boats and trains and their relation to rotational movements in dizziness and vertigo .......... 339
  Note on the autokinetic sensation .......... 347
Discussion .......... 348
  What is known about structural asymmetry? .......... 354
  The amount of functional asymmetry necessary to produce spiral movement .......... 361
  The significance of the drift of the spiral turns .......... 363
  The subjective element in spiral movement .......... 368
  The individuality of the paths .......... 372

The nature of the spiraling mechanism .......... 375
  Location .......... 375
  Relation of right-handedness and left-handedness to the spiral mechanism .......... 377
  Relation between spiral structure and spiral movement .......... 379
  Sizes of spiral turns in various organisms .......... 384
  Bilateral asymmetry in movement .......... 391
Summary .......... 392
Bibliography .......... 396

## INTRODUCTION

When a person loses his way in the forest or in snowstorms or fogs he goes around in circles as long as he keeps on moving, until in some way or other he finds his way again. The same is true of foxes, rabbits, antelopes,[1] and doubtless other animals, when hard pressed in the chase. Going down lower in the scale of organisms, where there are only poorly developed guiding senses, as in some of the crustacea, or none at all as in the protozoa and the flatworms, one finds these creatures going in circles all the time that they are in movement, but with a 'drift,' making the path a loose helical spiral. In all these animals and, in fact, in all motile organisms whatsoever, there is a deep-seated spiraling mechanism which leads an animal to go in a series of circles of spiral form when there are no guiding senses, as in the lower organisms, or when the guiding senses do not function through fear or for other reasons, in the higher.

Although a number of observers have studied special phases of spiral movement in the lower forms of life, especially in the ciliates and the flagellates, no extensive study has heretofore been made of this phenomenon in the higher animals. In fact, nothing was known of the presence of a spiraling mechanism in these forms, excepting folklore, regarding the going around in circles of lost persons or hunted animals, with the erroneous explanation based on asymmetry of the legs or of the body. And the experiments recorded in the following pages and in my book on Ameboid Movement were not undertaken to test experimentally the bit of folklore regarding paths made by lost persons.

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[1] See Harper's Magazine ('20, vol. 141, p. 430), where it is stated that antelopes run in circles when pursued with an automobile, up to their highest speed, which is about 97 kilometers per hour.

These experiments on man were suggested, on the other hand, by the sinusoidal path amebas make on a plane surface, which I noticed in 1917 when tracing a Mayorella bigemma for a few hours, merely to satisfy a curiosity as to what kind of path an ameba made in clear water on clean glass. The evident sinusoid resulting under these conditions suggested a projection of a helical spiral such as ciliates make on a plane surface—a path which the amebas followed in lieu of a helical spiral, because in their movement they are restricted to two dimensions of space. That the amebas should be 'compelled' thus to move in a sinusoid naturally aroused interest in the underlying causal mechanism and a curiosity to see how widespread the phenomenon might be. Some experiments on blindfolded persons were therefore tried, with the expectation that a considerable portion of the path would be sinusoidal. As it happened, however, the paths were clock-spring spirals, and only later was a minute portion of some paths discovered to be sinusoidal. The paths, however, were so regular, geometrically, that further data were collected as fast as time and facilities permitted. After a number of tracks were plotted, it became evident that the same mechanism which caused the clock-spring spirals in the experiments also caused the 'circles' when persons lost their way, for in the latter case the eyes had become useless as orienting organs for distance. And, similarly, wild game, when hard pressed, run in circles, because fear overcomes the orienting power of the eyes and nose and then the spiraling mechanism leads them in 'circles.' This investigation is therefore not only a contribution toward explaining a deep-seated and widespread biological phenomenon, but also an explanation for a bit of folklore about the paths of persons who lose their way and of game that is hard pressed in the chase.

The first experimental test of the presence of a spiraling mechanism in man was made on Johnson's race track at Knoxville, Tennessee, during the winter of 1917–1918. After a light fall of snow, several students and myself went out to the race track and each of us, in succession, tried to walk blindfolded to an object in the distance. The snow enabled us to examine the character of the track and to make notes, maps, and measurements. To our great surprise, each one of us walked in clock-spring spirals of a high degree of regularity. Were these paths helical spirals projected on a plane surface, perpendicular or nearly so to the long axes?

These results were interpreted as falling in line with the general hypothesis that a tendency to spiral locomotion is inherent in all moving organisms. An examination of biological literature shows,[2] as is well known, that the lower animals: flagellates, ciliates, rotifers, and the marine larvae of echinoderms, molluscs, worms, and crustacea swim in spiral paths, as well as some of the zoospores of algae and a few spermatozoa. So many organisms, in fact, swim in spiral paths that the negative aspect of the question is the more practical form for purposes of investigation: Are there any motile organisms which do not move in spiral paths when guiding senses are absent or not functioning?

With this question in mind, I examined a large variety of organisms, especially such as move in unusual ways, such as the free-living aquatic nematodes, the tadpole larvae of ascidians, salpas, young king crabs, several species of copepods, young marine annelid worms, together with some adults of fresh and salt water, small jellyfish, and a number of different kinds of spermatozoa. All of these were found to swim in spirals. But it needs to be added that some nematodes swim so poorly that frequently no forward movement occurs; but in these cases, although the bodily movements of the worms, including rotation on the long axis, are like those of the vinegar eel (Anguillula), for example, which is a very

[2] See Schaeffer ('20) and Bullington ('26) for a number of original observations and also for citations to earlier work.

good swimmer, no actual path is made. In sum, then, no forward-moving organism has yet been found that does not move in some form of spiral path when there are no orienting senses to guide it.

This extraordinary unanimity of observations on the prevalence of spiral movement among motile organisms can hardly be interpreted in any other way than that it is a universal property of moving living matter. In any case, it seems that less can be gained by continuing further to collect data to merely swell the number of organisms known to swim in spiral paths, than to study this phenomenon more intensively in certain groups of organisms that seem likely to yield more information on the working of the mechanism that causes or controls spiral movement. In fact, partly because of this belief, a considerable proportion of the time allotted to this work was devoted, from the very beginning of the discovery of the sinusoidal path in the ameba, to further study of unguided locomotion in man.

One of the first questions to arise in connection with spiral movement in man was whether the position of the axes of the organism had anything to do with the shape of the path. Would a person swim as well as walk a spiral path when blindfolded? And the further question was, Is any visible morphological character in man correlated with the shape of the path; that is, do right-handed persons walk in a certain direction, for example, and left-handed persons in the opposite direction, on account of better developed legs on one side or the other, as the case may be? And is it necessary for a person's motor mechanism to operate at all to go in spirals: that is, would a person drive an automobile in a spiral path when blindfolded? And again, where is the 'center' controlling the spiral mechanism located? These are some of the questions which the experiments described in the following pages were designed to answer.

Experiments such as those recorded in this paper obviously require close and sympathetic cooperation of a number of people and the expenditure of a large amount of time. And it needs to be mentioned, too, that the expenses were not inconsiderable, since suitable fields were always some distance off, requiring special transportation thereto, and the recording apparatus, a large camera, required expensive lenses. Fortunately, I was able to secure grants from the Bache Fund of the National Academy of Sciences, from the American Association for the Advancement of Science, and the Elizabeth Thompson Science Fund to defray most of the expenses incurred during the past six years. Without this aid, the investigation would have been greatly handicapped, and I consequently wish to express here my deep appreciation to the associations from which these grants were received, as well as to those persons who served as experimental subjects.

I am also deeply indebted to Mr. Alfred Ela for access to his large collection of citations on orientation and to Mr. Fred Allen, Jr., with whom I discussed various theoretical aspects of spiral movement, and to Dr. J. B. Eager for permission to work at the Topeka State Hospital. And finally I wish to thank those other friends of mine in physics and chemistry who have borne with me in the discussions of spiral movements in atoms, carbon chains, etc., for was it not one of the older mathematicians who warned us that "most of those who concerned themselves with spirals in the past finally turned insane?" But perhaps such a fate may be averted and my friends spared by keeping in constant contact with the spirals of Mother Nature, instead of the more ethereal types of the mathematicians, and thus, like Antaeus, derive renewed strength with each contact for further adventures in this field.

Be that as it may, I shall, nevertheless, take the risk to state at this point that I expect shortly to publish a paper on an experimental study of spiral movement in several species of amebas as a further contribution to an understanding of the underlying spiraling mechanism of motile organisms.

In the interest of accuracy and uniformity of meaning when conveying the concept of spiral movement from one language to another, and perhaps even in English, it might be well to use a technical term synonymous with 'spiral movement,' such as 'Spirokinesis,' which needs no translating. Under this term would be comprised the movement of organisms, spermatozoa, etc., in helical and clock-spring spiral paths while moving coordinately, when orienting senses are absent or not functioning.

### *Historical*

The belief that when persons who lose their way in the forest or in snowstorms or on the prairie they go "round and round in circles," is deeply ingrained in the folklore of many races and nationalities, especially those residing in northern latitudes where fogs and snowstorms abound. Among the Russians, Norwegians, Swedes, British Americans, American Indians, Chinese, Hindus, Filipinos, and negroes the belief is prevalent and occurs occasionally in the literature.[3] The same is true, doubtless, of many other races. I have been unable, upon inquiry and search, to find allusions to this belief in the ancient literature, but there is no reason to suppose that this phenomenon was not observed long ago in the history and prehistory of the race. In fact, it was only necessary that a person have the opportunity to lose himself and that he know what a circle or a clock-spring spiral is, to discover this phenomenon. It is likely, therefore, that this belief was known among the Aurignacians 20,000 years ago, for these people left behind them a reindeer antler with well-drawn conventionalized clock-spring spirals carved on it. This fragment of antler is the earliest record of a clock-spring spiral carved by man, and it therefore becomes probably the first entry in the bibliography of spiral movement in man.

The explanation of movement in circles by persons who have lost their way is stereotyped and almost as widespread as the belief that the circles occur. The explanation is invariably based upon an asymmetry of the body such as longer or stronger right or left legs, as the case may be, depending upon whether the persons circle to the left or to the right. This explanation has been reported by several writers on biological subjects (Thompson, '17, p. 492; Guldberg, '96, p. 811), and one of them (Guldberg) attempted to prove the point by measurements of bilateral asymmetry of the legs. But in spite of the evident interest that people have in this phenomenon, no actual path of a person moving in circles when lost or when blindfolded seems ever to have been recorded. The explanation for the phenomenon, though false, was so completely satisfying because of its simian simplicity that it must have seemed like wicked scepticism to investigate it by experimental methods. This is an excellent example of the persistent devastating effect of substituting logic for experiment.

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[3] Tolstoy, "Master and Man," "The Snowstorm"; Eugene O'Neill, "Emperor Jones"; Mark Twain, "Innocents Abroad"; popular song, "I'm going around in circles."

As has just been pointed out, the idea that there is some asymmetry in the legs or musculature of the body generally has been closely associated in the past with the belief that persons who lose their way go round in circles. Partly on this account, but also for other reasons, all kinds of the more striking structural asymmetries in man have excited interest and study. And even horses and birds and many other of the higher animals have been studied more or less from this point of view. The predominance of right-handedness over left-handedness in man has also been one of the standing puzzles throughout history. Likewise, the direction of the spiral twisting of the horns of sheep, goats, antelopes (compare p. 383), cattle, etc,, and of the shells of snails and other molluscs, of bean and other vines, fern spermatozoids, and great numbers of other animal and vegetable spiral structures have excited the curiosity and study of numerous naturalists. In fact, the prevalence of spiral structures in organisms is so extraordinarily widespread that in the discussion of these phenomena the simple assumption of bilateral asymmetry (unequal growth) has been held to place most of these structures, if not all of them, under this simple general explanation. A full discussion of the physiological phenomenon of spiral movement in man might, therefore, seem to call for discussion of all that has been written on spiral structures in organisms, seeing that writers have assumed a causal relationship between these two sets of phenomena. This literature is so extensive, however, and so widely scattered that the task would be very great, even if its utility were not doubtful. Especially is this the case since we have excellent evidence from the lower organisms that, although it may possibly be true that, at bottom, the same spiral mechanism produces both spiral structures and spiral movement, yet in a large proportion of animals the spiral mechanism does not go in the same direction in the same animal. That is to say, for example, a Paramecium aurelia is built on a right[4] spiral plan

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[4] There exists great confusion in descriptions referring to circular or spiral turning, although the matter is extremely simple: the object, person, animal, or path turns either to the right or to the left. There is no other way to turn. This describes it fully and completely. Such phrases as "from the right over to the left," "in the direction of the hands of a clock," "in a spiral like that of right-hand screw or corkscrew," etc., are unnecessarily long and some are of doubtful meaning. 'Clockwise' or 'anti-clockwise' means one thing to one person and the exact opposite to another. Thus Entz ('26, p. 433) uses 'clockwise' to mean right spiraling, the usual meaning; while Mast ('11, pp. 136, 147) uses 'anti-clockwise' to mean right spiraling. Again, there are not a few persons who, not having given any special thought to spiral turns, think that if one transferred the handle of an ordinary right-hand corkscrew to the other end, for example, it would then be left-handed.

There are only two possible ways of turning around in a circle (or rotating on an axis, providing, of course, there is progression or an outside reference point): one is right and the other is left. And all the world knows which way either direction is. Tell a traveler that at a certain point he has to turn to the right, and he has all the information he needs to know in which way to turn. And all the world is agreed on what is a right-hand screw. When one says, therefore, that Mr. A walked around in right circles or that Paramecium caudatum swims in a left spiral, the description of the direction is clear and complete, and the 'hay-foot, straw-foot' type of terminology should not be necessary.

The ease with which directions of spiral or circular turning confuses observers is illustrated in Conn's Algae of the fresh waters of Connecticut ('08, p. 67), where it is stated that the chlorophyll bands of the genus Spirogyra wind to the right. The figures, however, show eighteen species winding to the left and one to the right. All the filaments I have seen from a number of localities during the past year wind to the left. Conn may, however, have taken advantage of the ancient prerogative of botanists, to whom all left spirals are right and all right spirals are left. Even "an ordinary corkscrew," says Warming ('09, p. 33), (oral groove), but it moves in a left spiral path. In view of this situation, which is more or less general, it will be necessary to discuss only those books and papers which seem to be more directly concerned with these experiments on man.

### *Methods of work*

One of the first method-tests made was to determine whether seeing the records of paths made by another subject, or the subject's own previous paths, had any influence in walking subsequent paths. No such influence was detectable in straightaway paths, but in a few paths where the object was to walk around a circle, the knowledge that in one path the circles were smaller than the model, probably caused the circles in the next experiment to be much larger (N49, N50, pl. 5). Four subjects walked straightaways without knowing anything whatever of the object of the experiment; and these paths are indistinguishable from their subsequent paths, when they knew just what the experiments were about.

In the walking experiments the blindfolded subject wore a large hat or carried an umbrella to prevent sensing direction by sight or feel of the sun's heat. Sometimes a raincoat was wrapped around the head like a hood for the same purpose and also to prevent orientation to breeze. In a few trials the ears were plugged with cotton, but this was found later to be quite unnecessary. To keep the mind as uniformly occupied as possible, the subject was asked to keep in his mind's eye some object in the distance, toward which he was to walk and at the same time carefully count his steps. In some

"turns to the left for the botanists." On what scientific grounds the botanists include corkscrews among their subject-matter is not made clear, and I therefore am obliged tentatively to follow the rest of the world and the rule of priority, in resisting their classification, and shall continue to react to the corkscrew as if it were built on a right spiral plan. As a conclusion to this general argument on terminology, I think one may assume, without seeming unduly critical, that any casual statements occurring in the literature as to the right turning or left turning of any motion or structure needs checking (in addition to decoding from the botanists), for it is astonishingly easy to make a blunder even when one is especially studying the direction of turning.

experiments the subject was asked merely to walk forward without a definite objective and count by threes rapidly to keep the mind off everything else as far as possible. But very little definite difference could be observed in the paths made under these two conditions. The counting was done to make the conditions of the experiment as uniform as possible for the various subjects and to permit of verification. So far as could be ascertained, these expectations were fairly well met. Special details when necessary will be mentioned in connection with the experiments to be described in the following pages.

The walking was done on level fields of as large a size as could be found. While some of the observations were of such a nature that a field of 3 or 4 acres was of sufficient size, for most of the work a field of about 160 acres (65 hectares) was used. Even with this field the subjects occasionally came up against the fences. Although this field was as level as a floor, the experiments on other fields showed that this was not necessary, for considerable roll, including ditches and ridges and even low haystacks often make no perceptible difference in the curvature of the path. No orientation is possible by paying attention to the unevenness of the ground in an ordinary level field. Some experiments were also performed on ice on the large Wachusett reservoir in Clinton, Massachusetts, as a check. In a general way, it may be said that to a person walking under the conditions named, the outside world is cut off from him to an astonishingly high degree, and only a very strong steady breeze or an intensely hot sun near the horizon or a loud and continuous noise is capable of giving one a clue as to the direction in which he is walking. Of course, the subject was assured in all cases that notice would be given him to stop whenever he came near an obstruction of any kind, and after the first trial of any one subject, during which he acted more or less cautiously, the walking was done with confidence in a free and easy manner.

The experiments with automobiles were carried on in the same field in which the walking was done. The first few ex-on which the camera could be placed, and this is greatly to be regretted. But while it would be desirable for some purposes to have all camera records, it is not necessary in many cases. This is especially true when tests are made on right-hand turns as against left-hand turns, on the size of the circles walked, etc. For these and other purposes a free-hand record or map was made by first fixing several reference points in mind on the part of the horizon included in the subject's field. Then by noting on the path every hundredth step, a fair approximation of the correct path could be made.

Several of the paths made by the Ford automobile were recorded in the manner just described by a person on the ground, while the size of each of the circles was read off from the speedometer, as was also the speed, by a person in the car.

## EXPERIMENTAL OBSERVATIONS

The experiments in this paper are presented as far as possible in selected groups with reference to the particular method employed in carrying out the experiments. Each experiment as illustrated on the plates bears sufficient labeling to make clear whatever relationships it may have to other paths recorded in the paper. Each group as a whole is briefly described with reference to the general conclusions these experiments are believed to establish. In some cases these points involve discussion of earlier and different ideas by other writers, but such discussion will, for the sake of clearness, be reserved until the experiments have all been described.

The experiments are described somewhat after the manner of 'case records,' rather than by briefly summarizing or tabulating the results. This method was adopted partly because of the general interest in the data, but more particularly because of the marked individual differences between the various subjects, in which circumstance itself inheres one of the most interesting and important phases of behavior. A further reason for presenting all well attested details is that, since the underlying spiraling mechanism is not directly visible, its nature has to be inferred from its activities, following the methods of the physicists in studying the nature of the atom, and in this early stage of the work it is not possible to tell the important details from the unimportant.

### *Walking straightaway blindfolded*

In these experiments the subject was blindfolded and requested to walk straight ahead toward some tree or other object in the distance, counting his steps while walking. The object was to see whether there was any uniformity in the paths walked by any one individual and by different individuals. Walking straight ahead seemed to be the simplest condition possible for experimental purposes, and it seemed, moreover, to correspond more nearly with the path made by amebas, which suggested these paths in the first place, than any other form of path. In the illustrations the paths are identified by a letter and a number, the letter referring to the individual who walked the path, and this letter always refers to the same individual wherever it occurs in this paper, while the number is for identification of the particular curve. Where several curves occur in an evident series, the lower number refers to the curve walked first with the other numbered curves in order corresponding with the chronological sequence of the experiments. In all cases the beginning of the path is designated by a circle and the end by a cross. The straightaway paths aimed at an objective that corresponds to the top of the page directly upward from the circle of the particular path.

C. The first paths recorded in this series of investigations were published in my book on Ameboid Movement ('20, figs. 45, 46). In the spring of 1920, I was able to resume these experiments, and a set of paths is shown in the accompanying plate (pl. 1). The paths were walked by C, a university instructor, in the enclosure of a small race track at Knoxville, Tennessee. The small size of the enclosure did not permit of long paths, and many of the experiments had to be terminated prematurely because of obstructions in the way. The first two paths, *C1*, *C2* (pl. 1) turned to the right, initially. All succeeding ones, *C3*, *C4*, *C5*, *C6*, *C7*, *C8*, turned to the left. The first two paths were ordinary walking; the third one, *C3*, was slow running. In *C4*, C walked 25 steps, then stopped about 5 seconds, then walked 25 steps again, and so on. In *C5*, C walked to the left until he came to the fence, when he was halted. After he had taken the blindfold off his eyes he was asked to put it on again and walk back to his starting-point. The total time consumed in taking off and putting on the blindfold was about 15 seconds. For the sake of ready comparison with other curves of this kind, the first limb of the path is shown by a dotted line rotated about 90° to the right, so as to make the curve continuous throughout. It will be noticed that in this curve as in *C4*, the curvature of the path did not change markedly because of the interruptions. The curvature is carried over by the directing mechanism, as it were, although the motor mechanism had ceased functioning during these interruptions. The straight 'handle' to the first limb of this curve, as of the four corresponding curves, is not present in the second limb, and the dotted line shows that the convexity of the curve increases regularly, even over the interruption. But the 'drift' of the small spiral turns would be across the handle, as shown by the dotted line, which is contrary to the direction of the drift in uninterrupted paths (compare p. 339). After walking *C5*, three paths were made by walking backward, *C6*, *C7*, *C8*. In each case the path terminated at the fence. They all turned to the left. Each succeeding path was somewhat less convex than its predecessor, due probably to greater confidence and better coordination in walking backward blindfolded. But these paths are nevertheless much more convex than where the walking was forward, but this may be due partly to the fact that the backward steps are shorter than the forward steps.

Recalling now for a moment the conventional folklore explanation of going around in circles when lost, the question arises, Which leg was the stronger in this case, the right or

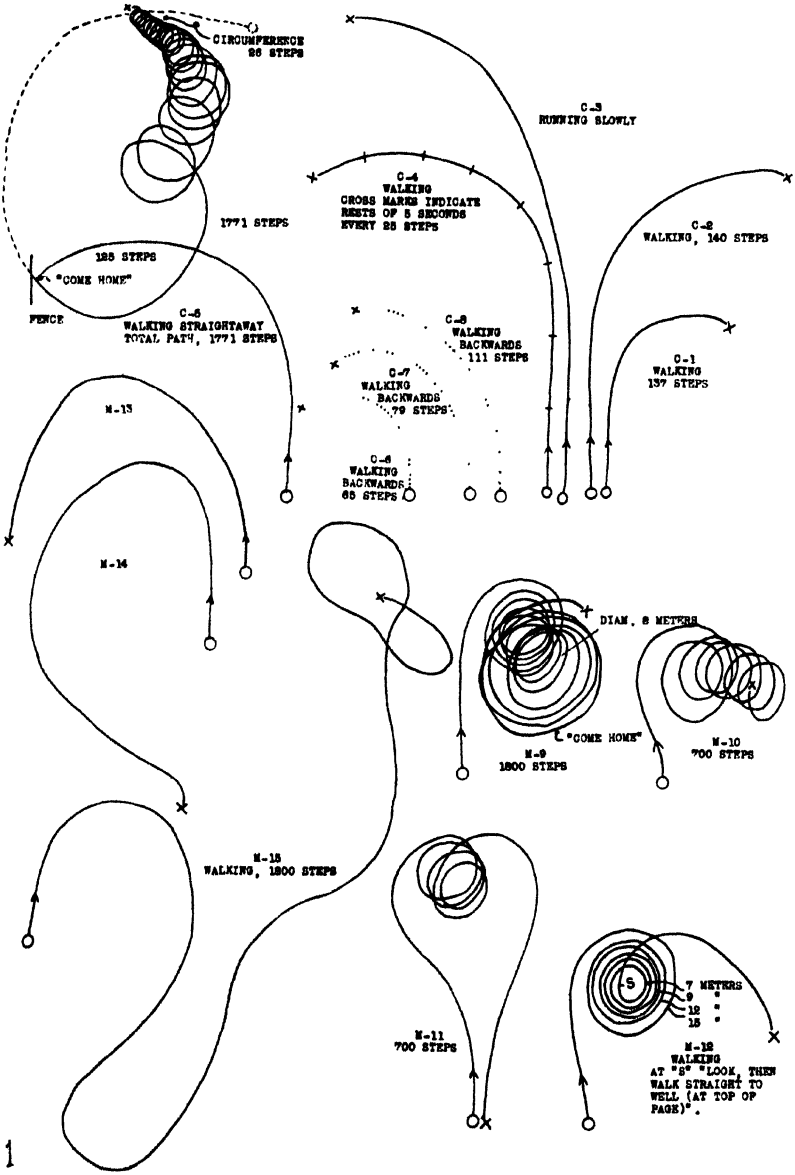
CIRCUMFERENCE
26 STEPS
1771 STEPS
125 STEPS
"COME HOME"
FENCE
C-5
WALKING STRAIGHTAWAY
TOTAL PATH, 1771 STEPS
C-3
RUNNING SLOWLY
C-4
WALKING
CROSS MARKS INDICATE
RESTS OF 5 SECONDS
EVERY 25 STEPS
C-2
WALKING, 140 STEPS
C-8
WALKING
BACKWARDS
111 STEPS
C-7
WALKING
BACKWARDS
79 STEPS
C-1
WALKING
137 STEPS
C-6
WALKING
BACKWARDS
65 STEPS
M-13
M-14
DIAM. 8 METERS
"COME HOME"
M-9
1800 STEPS
M-10
700 STEPS
M-15
WALKING, 1800 STEPS
M-11
700 STEPS
S
7 METERS
9 "
12 "
15 "
M-12
WALKING
AT "S" "LOOK, THEN
WALK STRAIGHT TO
WELL (AT TOP OF
PAGE)".
1

Plate 1

the left, seeing that there are both right and left turns?[6] And if strength alternated, what factor twice as powerful in producing the alternation was at work? Since backward walking is done with the right and the left leg changed about, side for side, as compared with forward walking, why are the curves in this series left, corresponding with the three preceding, instead of right as we might expect from the conventional explanation? Although these few experiments in themselves cast grave doubt upon the prevailing logic in this matter, the case is by no means complete, and a consideration of further experiments is necessary to show the complete absurdity of the conventional explanation.

M. It was my intention to continue these walking experiments during the regular session at the University of Tennessee with my three original subjects and others, but this was unfortunately prevented by the Fundamentalist eruption in this institution which made further comparative zoological research on man rather awkward there. In the spring of 1926, however, I was able to take up again the walking experiments in a large field near the University of Kansas, excellently suited for this purpose as well as for experiments in driving automobiles blindfolded. The first set of paths walked on this field are shown on plate 1, and were walked by M, a university student. Paths *M9* and *M10* were walked successively on the same afternoon. *M11* and *M12* were also walked on the same afternoon. *M11* is of very curious shape. It had to be terminated because of an obstruction. *M13*, *M14*, and *M15* were walked while the subject suffered from deafness in the left ear, due to a cold. What connection there may be between this fact and the peculiar shapes of the paths is not known. *M16* (pl. 2) is again a characteristic curve, with the circles becoming progressively smaller and then larger again. The latter feature, that is, a progressive increase in the diameter of the circles after a preliminary decrease, is characteristic of M's paths. *M17* is a sample of many paths which are altered by short preliminary 'training.' In this case M, blindfolded, turned around on his heel rapidly ten times to the right, then walked in a circle 3.5 meters in diameter to the right, then started off toward the objective in the distance. The general effect is to straighten out the latter section of the path. Other cases of this kind are recorded in those experiments where the object was to walk around a large circle.

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[6] The circles made by persons losing their way are of course much larger than these experimental circles with the eyes blindfolded; for with the eyes open it is possible to walk straight stretches as far as one can see clearly, which may be 10 or 20 meters in a snowstorm or in a fog, to several hundred meters in a forest. The circles in the latter cases are really irregular polygons and may have circumferences of 10 or even 30 kilometers, according to some accounts.

*M18* is a sample of a number of paths walked by different individuals to test directly the 'theory' that circles are due to a longer or stronger right or left leg. In this kind of path 50 steps were walked forward then the next 50 backward, then 50 forward again, and so on. The expectation would be, of course, a sinusoid on the asymmetry hypothesis. But a good spiral is the actual result with no change in curvature to distinguish it from a normal path walked forward, except that the sections walked backward are somewhat more curved, as was also the case in paths *C6*, *C7*, and *C8*. There is shown, however, a tendency to walk a sinusoid at the very beginning of some of these curves, as, for example, *N47* (pl. 5) and *Z56* (pl. 6). That the spiraling in *M18* is to the left seems to be due to the spiraling mechanism being 'thrown into gear' during the first 50 steps backward walking, which would be normally to the left, walking backward, for this subject. M walked backward with some difficulty. Further discussion of this kind of path will be found under *N47* and *Z56*.

*M20* is a path similar to *M18*, which was walked within 5 minutes after walking a long path going left around a 31-meter diameter circle. *M20* starts out 100 steps forward then walks backward, retracing his steps. The subject turned to the left at the beginning, probably because he had just previously been going around a left circle a large number of times. In the backward section he also walked left at first, but soon changed to his dominant direction, viz., right. This path illustrates the fact that the typical direction of turning is independent of whether one walks forward or backward. *M19* was a check path to test normality. It shows the characteristic decrease in size of the first few spirals, followed by an increase in the size. The final decrease in the size of the spirals was correlated with a sense of fatigue (compare *M44* and *M45*, pl. 5). *M21* is a path of an automobile which M directed by talking to the driver, without touching the steering mechanism. As in the other experiments, the object was to go in a straight path. M was blindfolded, sitting beside the driver and calling out directions in order to keep the automobile going in what seemed to M a straight path (compare *Z58*, pl. 6).

P. This subject, a university student, walked a large number of paths. Among them is a series of four paths which were walked to test the effect of breeze on spiraling. P walked north with a northwest breeze blowing, in *P22*. *P23* (pl. 3) was walked in the opposite direction. *P24* (pl. 2) was walked in the original direction, north, and *P25* (pl. 3) again in the opposite direction. The subject carried a raincoat over his head and stated that he was not conscious of orienting with reference to the breeze. As will be readily seen, the breeze had no effect on the diameter of the spirals, but it had a definite effect in causing the spiral to drift in the general direction of the breeze. *P26* is a path walked alternately forward and backward, with characteristic curvature.

This subject was frequently very sensitive to changes in breeze, character of ground, etc., while walking, and at those times the paths were very irregular, although always spiraling to the right.

### *Driving an automobile blindfolded*

The observations on trying to walk straightaway blindfolded proved that the spiral paths are not due to asymmetry of the legs, but that the mechanism which controls the spiral is situated either in the cord or higher up. To test this further, several persons were requested to drive a Ford coupé blindfolded straight toward some object in the distance.

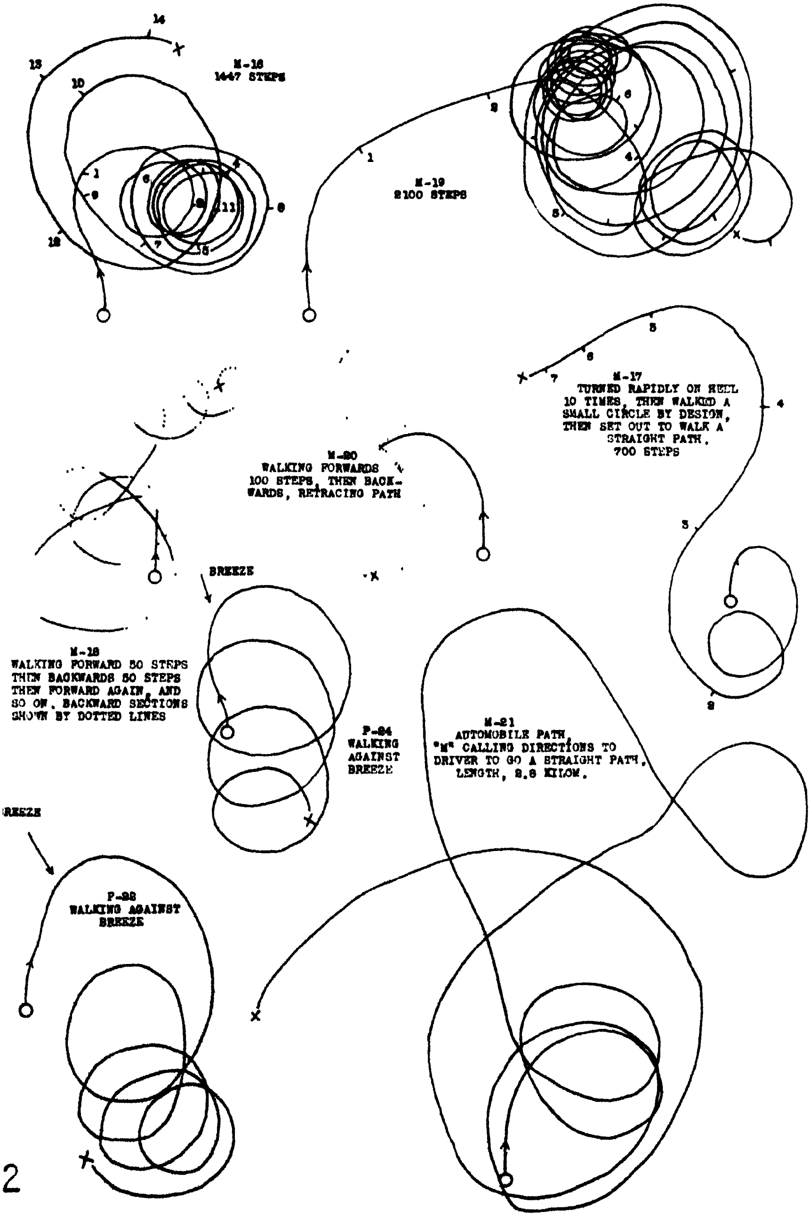

M-16
1447 STEPS
14
13
10
1
9
6
4
8
11
12
7
5
M-19
2100 STEPS
1
2
6
4
5
M-17
TURNED RAPIDLY ON HEEL
10 TIMES, THEN WALKED A
SMALL CIRCLE BY DESIGN,
THEN SET OUT TO WALK A
STRAIGHT PATH.
700 STEPS
5
6
7
4
3
2
M-20
WALKING FORWARDS
100 STEPS, THEN BACKWARDS, RETRACING PATH
BREEZE
M-18
WALKING FORWARD 50 STEPS
THEN BACKWARDS 50 STEPS
THEN FORWARD AGAIN, AND
SO ON. BACKWARD SECTIONS
SHOWN BY DOTTED LINES
P-24
WALKING
AGAINST
BREEZE
M-21
AUTOMOBILE PATH.
"M" CALLING DIRECTIONS TO
DRIVER TO GO A STRAIGHT PATH.
LENGTH, 2.8 KILOM.
BREEZE
P-23
WALKING AGAINST
BREEZE
2

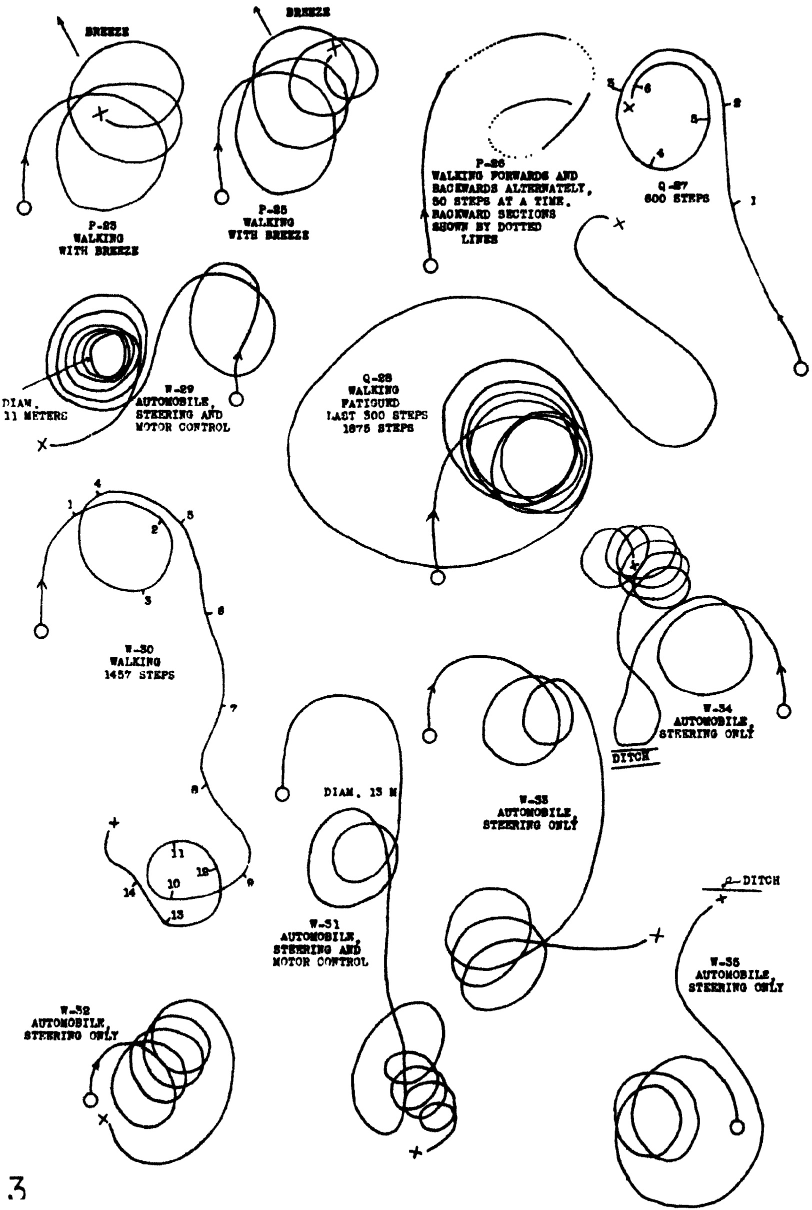
Plate 2

W. This subject is a university instructor and expert at driving automobiles. *W29* (pl. 3) is his first path in which he steered and controlled the motor. The smaller right-hand circles were about 11 meters in diameter. When this size was reached, as the circles became smaller, the steering mechanism of the Ford reached its limit of turning, and then W "knew that (he) must be going in circles," so he steered the Ford to the left. *W30* is a path walked soon after *W29*. *W31* is another path of the Ford steered and controlled by W. The first complete circle made was about 13 meters in diameter and the last series around 46 meters in diameter. *W32* is another path by the Ford, with W steering and another person controlling the motor. The path had to be terminated, since it led directly into an obstruction. *W33* is another path by the Ford, with W steering and another person in control of the motor. *W34* is a similar path, but is interrupted by running close to a ditch at the edge of the field. After the ditch was passed, the path was similar to the latter part of *W31* and *W33*. *W35* is another Ford path, with W steering and another person in control of the motor.

The paths of W and the Ford are remarkable for their general uniformity, consisting of a circle or two near the start, then a longer section with much less curvature, followed by a longer or shorter series of circles in spiral form. The first series of one or two circles was either to the right or to the left, but the final series was always to the right. The one walking path also has the same general characteristics as the automobile paths have. Another remarkable thing about these automobile paths is that they occupy generally only a little more space than the walking paths, and some of them less space than some of the walking paths. The speed of the Ford was about 13 kilometers per hour.

Q. *Q27* and *Q28* are two paths walked within forty minutes by a left-handed university instructor. Note the effect of fatigue in *Q28*.

X. The first path of this subject, a university graduate student, is an automobile path with another person control-


BREEZE
P-23
WALKING
WITH BREEZE
BREEZE
P-25
WALKING
WITH BREEZE
P-26
WALKING FORWARDS AND BACKWARDS ALTERNATELY, 50 STEPS AT A TIME. BACKWARD SECTIONS SHOWN BY DOTTED LINES
Q-27
600 STEPS
1
2
3
4
5
6
DIAM. 11 METERS
W-29
AUTOMOBILE, STEERING AND MOTOR CONTROL
Q-28
WALKING
FATIGUED
LAST 300 STEPS
1875 STEPS
W-30
WALKING
1457 STEPS
1
2
3
4
5
6
7
8
9
10
11
12
13
14
W-34
AUTOMOBILE, STEERING ONLY
DITCH
DIAM. 13 M
W-31
AUTOMOBILE, STEERING AND MOTOR CONTROL
W-33
AUTOMOBILE, STEERING ONLY
DITCH
W-35
AUTOMOBILE, STEERING ONLY
W-32
AUTOMOBILE, STEERING ONLY

3

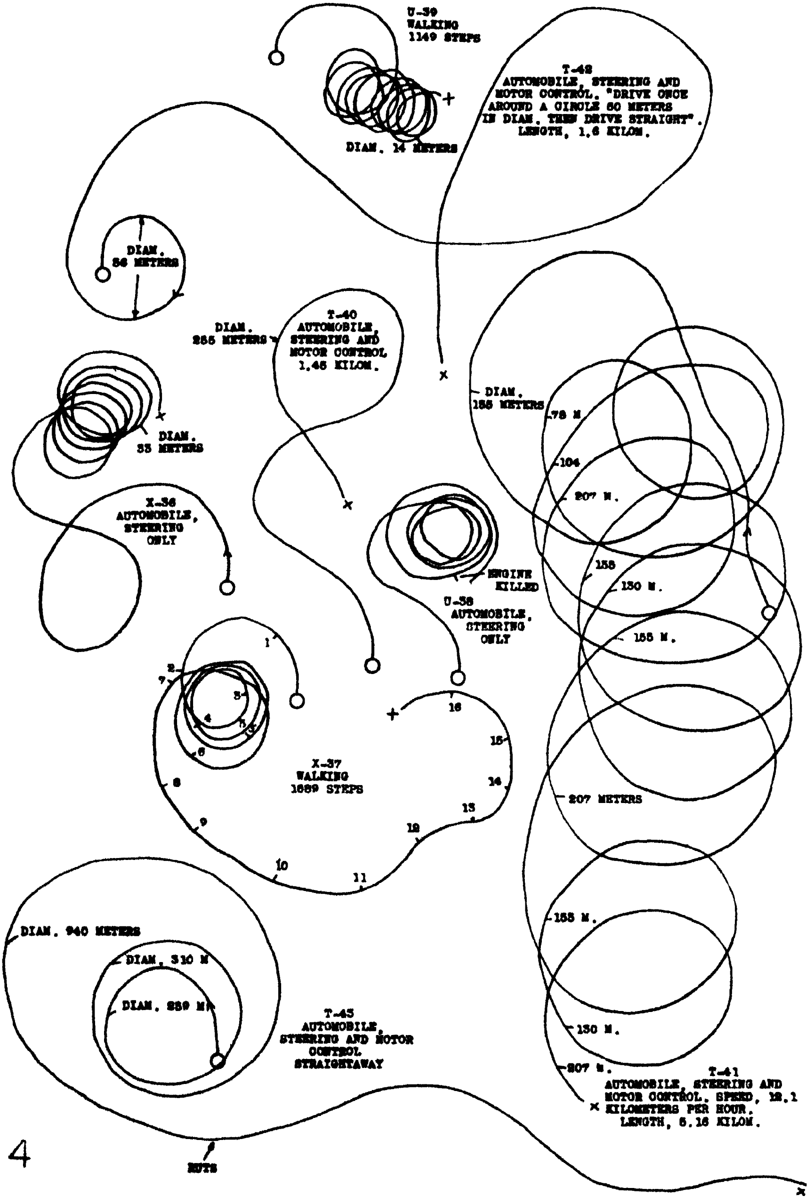
Plate 3

ling the motor, *X36* (pl. 4). The larger circles near the end of the path were about 33 meters in diameter. *X37* is a walking path in which the last three circles are also about 33 meters in diameter. In his third path X steered the Ford to the right for four circles, when the path had to be terminated.

U. This subject is a boy of nine years who had never steered an automobile or motorboat. He steered a Ford blindfolded with another person controlling the motor as shown in *U38* (pl. 4). *U39* is a walking path of 1149 steps. The circles in the walking path were about 14 meters in diameter, while the smaller circles in *U38* were about twice this diameter.

T. *T40* is the first path made by a university graduate student on the open prairie in western Kansas and eastern Colorado, by driving a Ford (touring) blindfolded toward a little speck on the horizon, probably a water tower. *T41* (pl. 4) is his second path with ten circles arranged in spiral form, the total distance being 5.16 kilometers. The diameter of the circles varied from 75 meters to 207 meters. *T42* is a path of the Ford driven around an imagined 60-meter diameter circle to the right. When T thought he was around, he drove off straight to the water tower in the far distance (toward top of page). Very soon he steered into a left circle, bringing up against an obstruction as the speedometer registered 1.6 kilometers. The next path was the result of driving straight for the water tower, *T43*. Three left circles totaling 4 kilometers were made. Unfortunately, I neglected to have one or more walking paths made by this subject for comparison. The average speed of the Ford in these experiments was 12 kilometers per hour.

The Ford paths by X, U, and T agree in general with those of W as to size and correspondence with the walking paths of the same persons (X and U). In addition, it may be noted that the series of circles are frequently in spiral form, that is, there is a successive drift to the successive circles, just like the walking circles are. This resemblance is very remarkable, seeing that the spiral character cannot be ascribed


U-39
WALKING
1149 STEPS
DIAM. 14 METERS
T-42
AUTOMOBILE, STEERING AND
MOTOR CONTROL. "DRIVE ONCE
AROUND A CIRCLE 60 METERS
IN DIAM. THEN DRIVE STRAIGHT".
LENGTH, 1.6 KILOM.
DIAM.
56 METERS
DIAM.
255 METERS
T-40
AUTOMOBILE,
STEERING AND
MOTOR CONTROL
1.46 KILOM.
DIAM.
33 METERS
X-36
AUTOMOBILE,
STEERING
ONLY
DIAM.
155 METERS
78 M
104
207 M.
158
130 M.
155 M.
ENGINE
KILLED
U-38
AUTOMOBILE,
STEERING
ONLY
1
2
7
3
4
5
5
6
8
9
10
11
12
13
14
15
16
X-37
WALKING
1689 STEPS
207 METERS
155 M.
130 M.
207 M.
T-41
AUTOMOBILE, STEERING AND
MOTOR CONTROL. SPEED, 12.1
KILOMETERS PER HOUR.
LENGTH, 5.16 KILOM.
DIAM. 940 METERS
DIAM. 310 M
DIAM. 289 M
T-43
AUTOMOBILE,
STEERING AND MOTOR
CONTROL
STRAIGHTAWAY
RUTS

4

Plate 4

to asymmetry in the Ford, since spirals are made either to the right or to the left; but the spiral character must be due to the spiraling mechanism in the person steering, which, as these experiments show, must certainly reside above the shoulders. See also *Z57* and *Z58* (pl. 6). A 'control' path with an unsteered Ford showed no spirals.

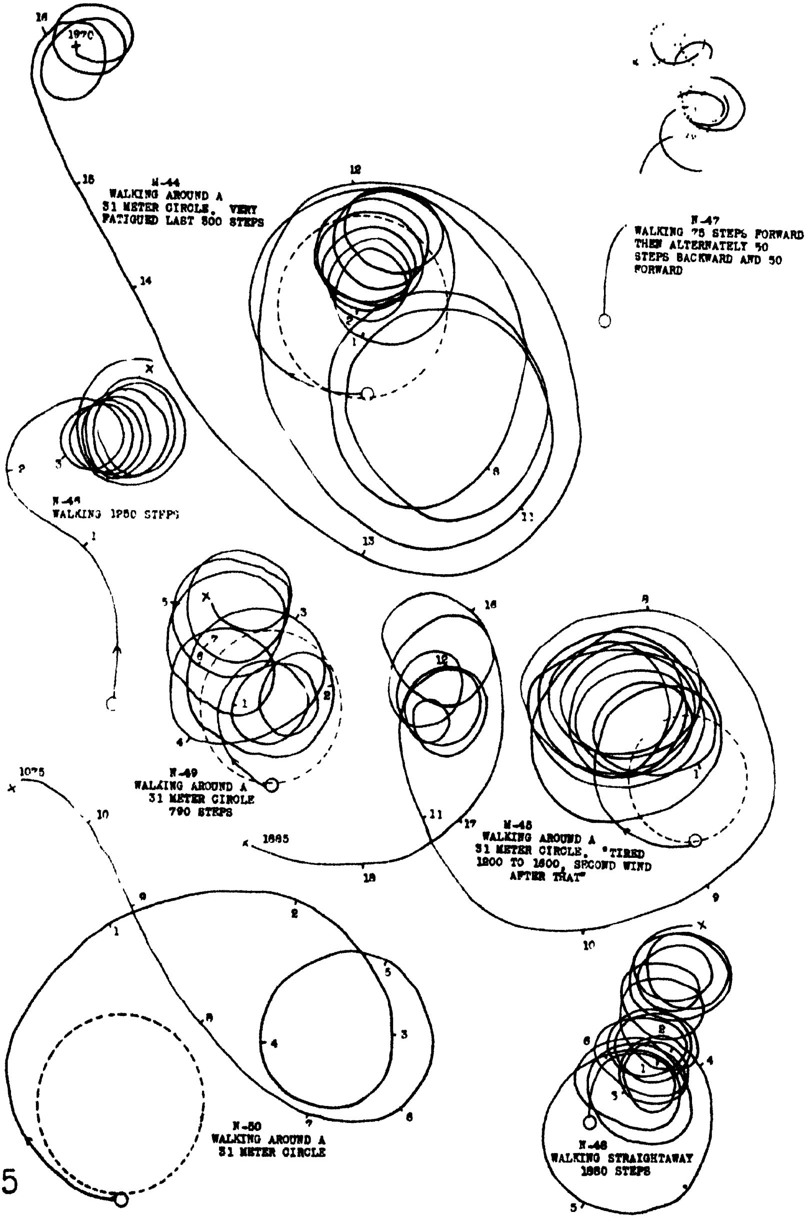
It would be exceedingly interesting to carry out similar experiments with airplanes, but so far no good opportunity to do so has presented itself.

### *Walking around imagined circles*

The experiments described thus far all had, as their objective, walking or driving straight ahead toward some distant object, with the result that the path soon changed into a series of circles of an average diameter of about 33 meters, usually arranged in the form of a spiral. The question now arises, What kind of path does a person make who walks deliberately around an imagined circle, blindfolded, of about 31 meters' diameter?

M. *M44* (pl. 5) is a path walked around a 31-meter circle marked off with small wisps of hay. The first circle was a little larger than 31 meters in diameter, then followed seven circles much smaller than the model circle. Then followed two circles a little larger than the model, then another considerably larger. Then the path is almost straight for 200 steps. At the end of the path there are four circles of about 31 meters' diameter. The subject said he felt very fatigued the last 300 steps.

*M45* is a similar path. Seven circles of about the size of the model came first, followed by a large semicircle about 185 meters in diameter. Then came six circles averaging about 23 meters in diameter, which were followed by a long slightly curved stretch of path. The subject reported that he was "tired from steps 1200 to 1600, then got second wind." This subject walked another similar path soon after *M45*, which began with seven circles about 31 meters in diameter, fol-


16
1970
15
14
13
12
11
8
2
1
N-44
WALKING AROUND A
31 METER CIRCLE. VERY
FATIGUED LAST 500 STEPS
N-47
WALKING 75 STEPS FORWARD
THEN ALTERNATELY 50
STEPS BACKWARD AND 50
FORWARD
N-46
WALKING 1950 STEPS
N-49
WALKING AROUND A
31 METER CIRCLE
790 STEPS
N-45
WALKING AROUND A
31 METER CIRCLE. "TIRED
1200 TO 1600, SECOND WIND
AFTER THAT"
1885
1075
N-50
WALKING AROUND A
31 METER CIRCLE
N-48
WALKING STRAIGHTAWAY
1980 STEPS

5

Plate 5

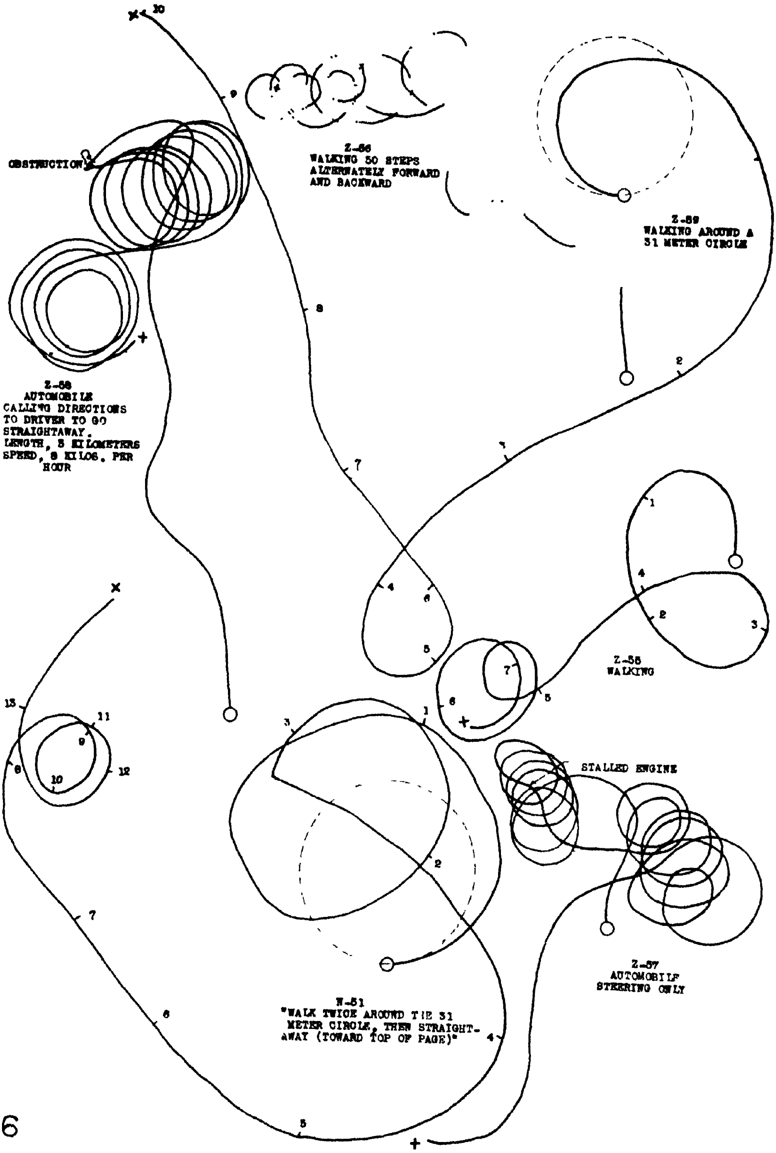
lowed by a semicircle about 160 meters' diameter. Then came a circle about 45 meters' diameter which corresponded with momentary sense of fatigue. Then followed two large circular loops of about 78 meters' diameter, then three small circles about 16 meters in diameter, then a long open curve against an obstruction.

N. This subject is a girl, graduate student and left-handed. Her first path, *N46* (pl. 5) was to be a straightaway to an object in the distance. She walked a typical right-hand curve with a somewhat more pronounced 'heterotropic' (i.e., both right- and left-curving sections in it) handle at the beginning of the path that is usual where this feature occurs at all. Soon thereafter she walked another path, *N47*, which consisted of 75 steps forward, then alternately 50 steps backward, and 50 forward toward an object in the distance. This path resulted in a right-hand spiral, with the first forward section veering to the right, the first backward to the left, the second forward to the right, and all following sections to the right. *N48* is another straightaway path. It fell at once into a spiral of thirteen small circles, averaging about 28 meters in diameter, and one circle about 71 meters in diameter.

*N49* is a path around an imagined 31-meter circle consisting of eight circles somewhat less than 31 meters on the average. *N50* was walked soon after. It is a very open curve with two circles, a long stretch almost straight and a beginning of a definite curve to the left. In *N49* the subject thought she "was going at a tangent at times." The more open *N50* is perhaps due to a tendency to correct for her smaller preceding curves, for she had seen the drawing of *N49* before she started on *N50*.

*N51* (pl. 6) was designed to test the effect of left-hand circling on a person who normally makes a right-hand spiral.

The subject was asked to walk twice around an imagined 31-meter circle to the left and then start off straight to a definite object in the distance (which would actually have been at the top of the page). Two complete circles of course brought the subject to the point of starting, and then a 90°


10
9
OBSTRUCTION
Z-56
WALKING 50 STEPS
ALTERNATELY FORWARD
AND BACKWARD
Z-59
WALKING AROUND A
31 METER CIRCLE
8
Z-58
AUTOMOBILE
CALLING DIRECTIONS
TO DRIVER TO GO
STRAIGHTAWAY.
LENGTH, 3 KILOMETERS
SPEED, 8 KILOS. PER
HOUR
2
7
1
4
6
5
Z-55
WALKING
13
11
9
8
10
12
3
1
7
6
5
STALLED ENGINE
2
7
Z-57
AUTOMOBILE
STEERING ONLY
N-51
"WALK TWICE AROUND THE 31
METER CIRCLE, THEN STRAIGHT-
AWAY (TOWARD TOP OF PAGE)"
6
4
5

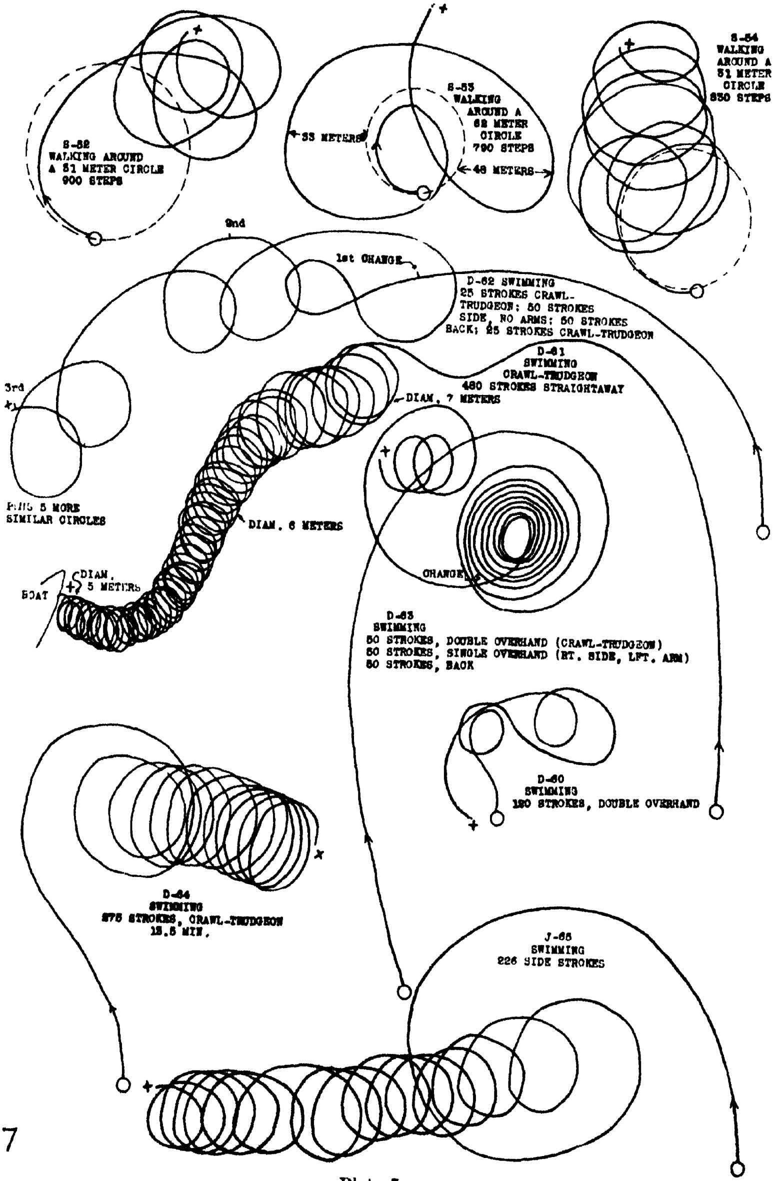
6

Plate 6

turn on the heel to the left was required to face in the direction of the objective. The interesting thing about the curve is that the path, after the two circles were supposed to be completed, began to curve only slightly to the right at first then more strongly, followed by a straight stretch of about 250 steps. The straight section led into two right circles about 78 meters in diameter, which were followed by an open right curve about 100 steps to an obstruction.

Z. *Z59* (pl. 6) is a path walked around an imagined circle of 31 meters' diameter by a university instructor. It is a very open curve with one left circle about 78 meters in diameter in the middle of it, followed by an almost straight path 350 steps long to an obstruction. Several other paths are given for comparison. *Z56* is a path of a series of forward and backward walking sections of 50 steps each, toward an object in the distance. The first three forward sections veered to the right and the first three backward sections to the left, thus producing an irregular wavy path. But with the fourth forward section a series of circles in spiral form was laid down, without any further evidence of waviness in the path. *Z55* is a straightaway path with one left and two right circles in it. *Z57* is an automobile straightaway path, Z steering and another person controlling the motor. Two series of left circles separated by a pause due to slight motor trouble, followed by a long open S-curve to an obstruction, made up this path. *Z58* is a path of an automobile which Z, blindfolded, directed to go in a straight line by calling directions to the driver. The circular path shows that the spiraling mechanism functions even when the person who does the guiding has no contact with the steering mechanism whatever. Similar results are also obtained on boats and trains (p. 339).

S. To throw further light on the largest size of imagined circle one can walk around with any degree of accuracy, a graduate student, S, was asked to walk around an imaginary circle 31 meters in diameter, then around a 62-meter circle, then around a 31-meter circle again. The resultant paths are shown in *S52*, *S53*, and *S54*, respectively (pl. 7). The same


S-52
WALKING AROUND
A 51 METER CIRCLE
900 STEPS
S-53
WALKING
AROUND A
62 METER
CIRCLE
790 STEPS
33 METERS
48 METERS
S-54
WALKING
AROUND A
51 METER
CIRCLE
850 STEPS
2nd
1st CHANGE
D-62 SWIMMING
25 STROKES CRAWL-TRUDGEON; 50 STROKES SIDE, NO ARMS; 50 STROKES BACK; 25 STROKES CRAWL-TRUDGEON
D-61
SWIMMING
CRAWL-TRUDGEON
480 STROKES STRAIGHTAWAY
DIAM. 7 METERS
3rd
PLUS 5 MORE
SIMILAR CIRCLES
DIAM. 6 METERS
DIAM.
5 METERS
BOAT
CHANGE
D-63
SWIMMING
50 STROKES, DOUBLE OVERHAND (CRAWL-TRUDGEON)
50 STROKES, SINGLE OVERHAND (RT. SIDE, LFT. ARM)
50 STROKES, BACK
D-60
SWIMMING
180 STROKES, DOUBLE OVERHAND
D-64
SWIMMING
875 STROKES, CRAWL-TRUDGEON
13.5 MIN.
J-65
SWIMMING
226 SIDE STROKES

7

Plate 7

experiment was tried by two other persons with similar results. These results correspond with the general observation on walking straightaway when the path changes into a series of circles of nearly uniform size; that is, the largest size of such series of circles (spirals) is around 31 meters in diameter. But circles of this size are, of course, not as numerous as smaller circles, since these would be at the maximal end of the size series.

### *Swimming straightaway blindfolded*

In all the experiments recorded thus far the long axis of the person performing was perpendicular to the path walked or driven at any given point of the path. The question naturally arose whether the character of the path would change if the body axis was laid, for example, parallel with the path, as would be the case in swimming. To test this point a number of records were made of blindfolded swimmers swimming straightaway. To secure accuracy in the records, the path of each swimmer was traced on transparent paper laid on the ground-glass of a camera 60 cm. by 90 cm., especially constructed for the purpose. The maximum amount of accuracy was thus obtained from all points of view, except one, viz., it was very difficult to make exact allowance for a slight drift in the water due to tidal action or breeze. The drift was insufficient to change the path in important particulars, the chief effect being to shorten or lengthen slightly a series of circles forming a spiral, if the long axis lay in the direction of the drift. The effective drift varied from 30 cm. to 120 cm. per minute.

Each path is drawn to scale, except where otherwise indicated, when for some reason the camera was not available. In some paths, however, the scale is different from that of the others, owing to the large area covered. These paths, although drawn to scale individually, can therefore not be directly compared as to size with the other records. But an accurate comparison may nevertheless be made upon the basis of the number of swimming strokes, which is given for each path.

As might be expected, the same swimming stroke was not used by all the swimmers, and no attempt was made to have uniformity in this respect. In fact, many of the experiments were designed so that several different strokes were employed at stated intervals, for various reasons, among these being a desire to change the shorter axes of the body with reference to the direction of the path as well as the longer axis. Swimming on the back thus requires a reversal of the dorsoventral axis from the position taken in swimming the breast or trudgeon strokes. And in the side stroke there is again a different position of the shorter body axis. If, therefore, the positions of the axes of the body have any effect on the character of the path, the various swimming strokes should bring it out.

D. The path *D60* (pl. 7) was made by a premedical student and a good swimmer. The stroke was a combination of crawl and trudgeon. The path was 120 strokes long and ended against an obstruction. The path was in part recorded on a camera, the rest being added to it from a free-hand sketch. *D61* was made almost immediately following *D60*. It is a free-hand sketch combined from two sketches made independently by different persons. This is a very remarkable path, because of the large number (fifty-two) of small spiral turns. The spiral turns were about 7 meters in diameter at the beginning and gradually decreased in size until the end of the series, when they were only about 5 meters. The downward bend in the path just before the spiral turns begin is a very rare phenomenon and its significance is not entirely clear. When this path is compared with several other paths made by this subject, it appears as if this downward bend might represent a beginning tendency to form a right spiral like that in *D64*, but for some reason was swung back soon again into a left spiral, the dominant direction of turning for this subject.

*D63* was recorded on a camera and is an exact reproduction of the path, except that the nine small circles in the center of the first series were actually piled up on each other in a slightly irregular manner. In the drawing they are drawn parallel, in order to show the places where changes of swimming stroke were made. The first 50 strokes were of the crawl-trudgeon type, the next 50 were single overhand (right side), and the last 50 were on the back. The change of stroke did not perceptibly change the curvature of the path, unless it is assumed that its action was delayed in the second change. The back stroke was not as smooth as the other two strokes.

*D62* is the first part of a path made ninety minutes after *D64*. It is a left spiral and resembles *D61* somewhat. In this case a change was actually made into a right turning spiral, but it was immediately changed back into a series of nine left circles. Whether the first change of stroke caused this one right turn cannot be definitely assumed. A similar change did not produce any noticeable effect in *D63*, but that was in a right curve, and three right turns had already been made. This seems to make it more difficult for a change of curvature to occur than if no turns have been made. In this curve the change to the back straightened out the curve after the next turn, just as it did in *D63*. But the third change (not shown) to crawl-trudgeon had no perceptible effect.

*D64* is remarkable for its regularity. Its beginning section is of the typical heterotropic type. The last ten turns of the spiral are all of almost exactly the same size—about 21 strokes in circumference. There was a slight breeze in the direction of the axis of the spiral which may have stretched it out about one-half the diameter of a spiral turn. It should be noted, too, that the heterotropic stem consists of almost equal segments of a left and a right circle, if one selects a point near the top of the stem for the upper end of the right segment. Whether this feature of the path represents actually a balance between a tendency to turn to the right and a tendency to turn to the left, alternating, cannot be determined, owing to too few such paths. But, as far as the records show, the question-mark type of curve occurs more frequently in individuals who walk both right and left spirals.

J. *J65* (pl. 7) is a path made by a girl, a college instructor. This spiral compares with *D64* in its regularity, but there is no sign of any right-turning at any point. The diameter of the spiral turns is about 6 meters and is very uniform throughout. This path, as all the other swimming paths, started off with a dive, but in this case, although the distant objective was faced squarely while standing on the retaining wall, yet when the bathing cap was pulled over the eyes just previous to the take-off, the dive itself veered definitely to the left.

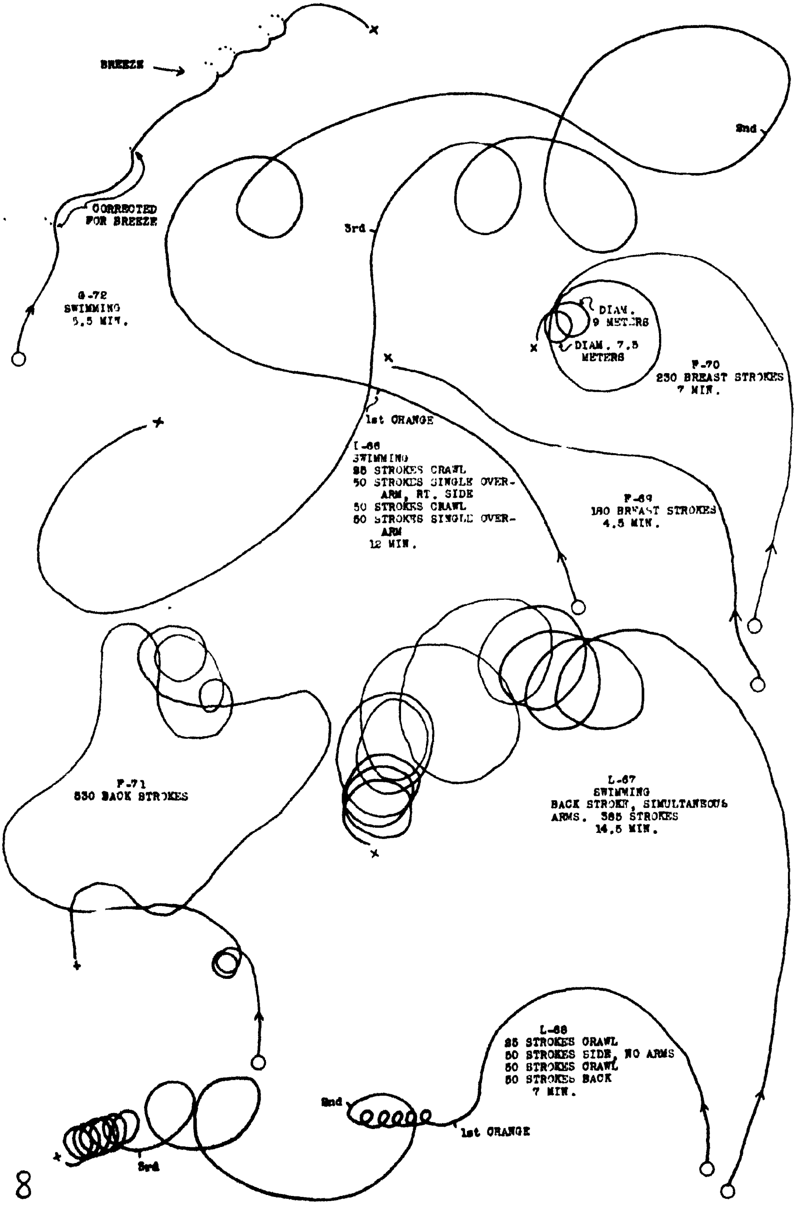
I. An art student and excellent swimmer, a girl, swam path *I66* (pl. 8). It is an extremely open curve with the question-mark type of start. One right and three left circles were made.

L. Paths *L67* and *L68* (pl. 8) were made by a college girl. The first path, *L67*, is of large size, the spiral turns averaging 14 meters in diameter, while the stem of the path is over 122 meters long, that is, from the origin to the beginning of the first circle. The spiral is somewhat irregular, excepting the last three circles, which may indicate the beginning of fatigue, although fatigue was not reported. *L68* is very remarkable because of the five very small left circles which were made soon after the first change of stroke. The diameter of these circles was between 2.1 and 2.5 meters. It is the smallest uniform swimming spiral of which I have any record. The final part of the course was made with a back stroke, and the spiral turns there were about 4.5 meters in diameter. This path as a whole is remarkable in that each change of stroke is associated with a change in the character of the path, although all of the spiral turns are to the right. But the beginning of the path veers to the left and the drift of the spiral is to the left. This drift is independent of the slight tidal drift. This path was made after *L67* and several smaller paths, all of which turned to the left and ended against obstructions before anything of importance was observed and recorded. It is possible, therefore, that the peculiarities of this path are due to the beginnings of fatigue. No fatigue was reported, but no special examination was made of this point, since the correlation of the onset of fatigue with the appearance of a series of smaller circles in the path was not discovered until later.

F. F, a girl research assistant at Cold Spring Harbor, swam *F69, F70,* and *F71* (pl. 8), together with several others unrecorded here, which resemble *F69*. The paths are strikingly individual and are remarkable for the long open stretches in them and the absence of spirals of more than two or three turns. The small spiral turns at the end of *F70* may have been brought on by fatigue, as may also several circles in the middle of *F71*. No fatigue was reported, but here again no special examination was made on this point. All the circles in these paths turn to the left. As for the rest of the parts of the paths, the long stretches of more or less irregular curves, there is no adequate explanation of these stretches apart from a probable connection with temperament or sensitiveness to external stimuli (compare p. 372).

G. A college girl and research assistant at the Carnegie Laboratory swam *G72* (pl. 8), and a number of other paths unrecorded here. It is of interest because of the evident corrections which the swimmer made at about six points in the path. There was a slight breeze which whipped up ripples on the water, and as she turned to her left in the direction of the breeze she unconsciously corrected her path and turned to the right. But in each case except the last she veered to the left again. The distances between the corrections grew shorter as she progressed and the curve of the path just before each correction was more convex in the last few corrections than in the first two. The several other paths made by the same subject are open irregular curves somewhat more irregular than *F69*, with no circles in them. The irregularity of these paths were also very probably due to unusual sensitiveness to external factors (compare p. 372).

E. Next is a series of ten swimming paths made by a university graduate student and research assistant. The first


BREEZE
CORRECTED FOR BREEZE
G-72
SWIMMING
5.5 MIN.
2nd
3rd
DIAM.
9 METERS
DIAM. 7.5
METERS
F-70
230 BREAST STROKES
7 MIN.
1st CHANGE
L-66
SWIMMING
25 STROKES CRAWL
50 STROKES SINGLE OVER-
ARM, RT. SIDE
50 STROKES CRAWL
50 STROKES SINGLE OVER-
ARM
12 MIN.
F-69
180 BREAST STROKES
4.5 MIN.
F-71
530 BACK STROKES
L-67
SWIMMING
BACK STROKE, SIMULTANEOUS
ARMS. 385 STROKES
14.5 MIN.
L-68
25 STROKES CRAWL
50 STROKES SIDE, NO ARMS
50 STROKES CRAWL
50 STROKES BACK
7 MIN.
2nd
1st CHANGE
3rd

8

Plate 8

five paths, *E73, E74, E75,* and *E76* (pl. 9), were all made on one and the same afternoon. In three of the paths the spiraling is to the right, and in one case *E76,* the initial turn is to the left, but the turn was not completed owing to an obstruction. This is the only case of turning to the left for this subject. But in the other three cases, although the spiraling was to the right, it got very soon into left territory, which is quite different from what occurred in all his other paths except one, *E79,* where the stroke was changed. It is also to be noted that the path *E73* starts with a typical heterotropic shape, which seems to be correlated with a balance between right and left tendencies in turning. In comparing these four paths with the other six, which were made on two other days, one can easily see that a definite right-turning tendency was operating throughout these six and there are no heterotropic starts present. This subject was therefore nearly 'on a balance' between right and left turning on the day when the first four paths were made and decidedly right turning on the two days when the other paths were made.

But there is also a marked difference in the group of six paths between the three that were made on the 23rd of August and the three not recorded here made on the 25th. In the three of the 25th the spirals all move rapidly off to the right, while the spiral turns of the paths made on the 23rd, *E77, E78,* and *E79* were made on the same spot. The change of stroke in *E79* probably lengthened the spiral turn unduly to the left.

H. *H80* (pl. 9) is one of seven paths made by a research assistant at Cold Spring Harbor. This subject made paths very similar to those of G, but they were on the whole freer from sharp curves. H's paths ranged from 80 to 140 strokes in length, consisting of wide sweeps which never made as much as a semicircle anywhere along their lengths. And there is no evidence that this subject corrected his paths unconsciously because of differences in breeze or light. The subject did not report noticing any differences in stimuli in which he might have reacted. Whether the circles at the end

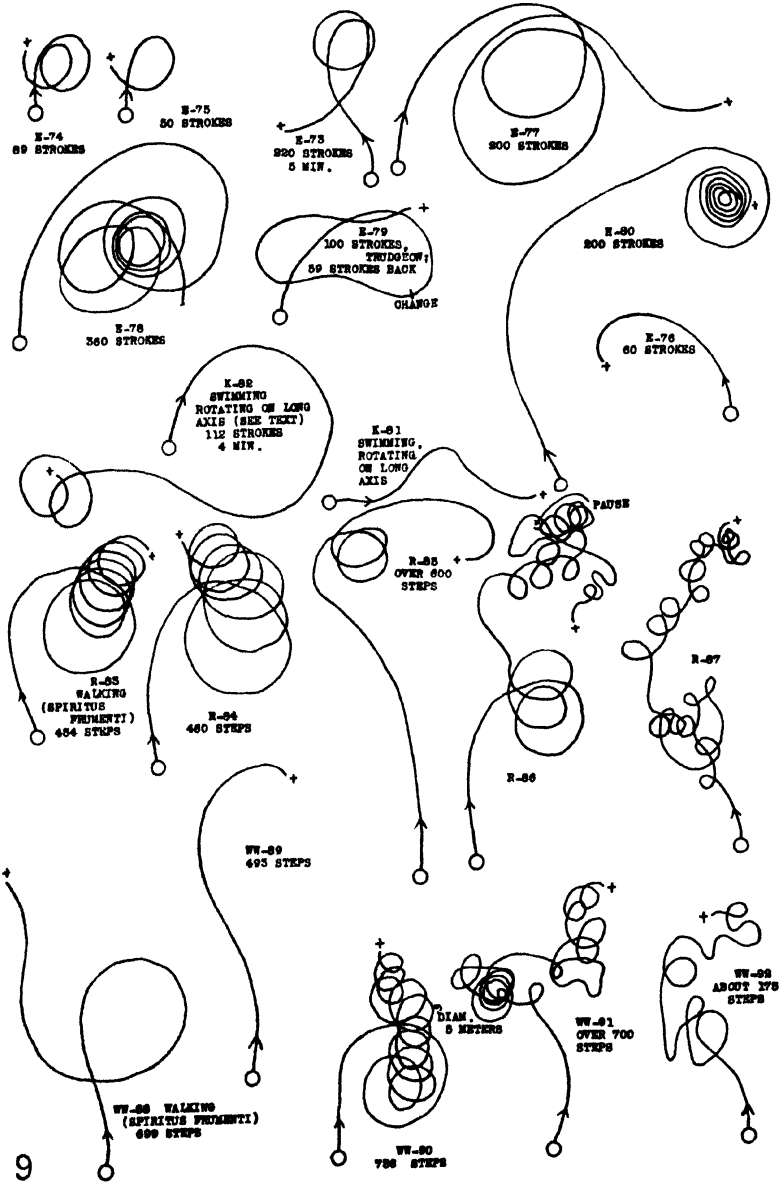

E-74
89 STROKES
E-75
50 STROKES
E-73
220 STROKES
5 MIN.
E-77
200 STROKES
E-78
360 STROKES
E-79
100 STROKES,
TRUDGEON;
59 STROKES BACK
CHANGE
E-80
200 STROKES
E-76
60 STROKES
K-82
SWIMMING
ROTATING ON LONG
AXIS (SEE TEXT)
112 STROKES
4 MIN.
K-81
SWIMMING,
ROTATING
ON LONG
AXIS
PAUSE
R-85
OVER 600
STEPS
R-83
WALKING
(SPIRITUS
FRUMENTI)
454 STEPS
R-84
460 STEPS
R-87
R-86
WW-89
493 STEPS
WW-88 WALKING
(SPIRITUS FRUMENTI)
690 STEPS
WW-90
736 STEPS
DIAM.
5 METERS
WW-91
OVER 700
STEPS
WW-92
ABOUT 175
STEPS
9

Plate 9

of path *H80*, which were from 6 to about 8 meters in diameter, were due to fatigue is improbable, since this is the first path made on that day.

K. Although it has been brought out in a number of these records that a difference in the swimming stroke does not change the general character of the path and that therefore the shorter axes of the body play no appreciable part in the curvature of the path, yet there was one more set of conditions with respect to axes positions that was experimentally practicable which had not yet been tried. This experiment consisted in rotating on the long axis of the body continuously while swimming blindfolded through the water straightaway. One of the boys in the laboratory, K, who had previously instructed swimming classes, volunteered to make this rather difficult experiment. To accomplish the rotation he made first a crawl stroke, then a single over-arm on the right side, then a back stroke, and then a single over-arm on the left side. Each series of four such strokes rotated the swimmer once on the long axis. The first attempt, a 'practice path,' is a three-minute path shown in *K81* (pl. 9). *K82* is his second path, of four minutes, consisting of one right turn and a short spiral of two left turns at the end of the path. The path was terminated because of slight dizziness due to the rotation. There were about 28 complete rotations of the body in this path. Unfortunately, no other records in which a single stroke was used by K were made, so no comparisons of that sort are possible. Nevertheless, it seems impossible to conceive that the semicircular canals could have a point of reference within themselves with which to bring about such smooth circularity as is here observed, for this kind of rotational swimming requires not only the quick and extensive movements of the head seen in ordinary swimming, but continuous rotation of the head in addition while these movements are going on.

### *Effect of a drug on spiral movement*

All the experiments recorded so far in walking, automobile driving, and swimming were made upon the normal neuromuscular system; no attempt was made to change its activities in any way except as these might be changed by fatigue or a particular stroke used in swimming, etc. There are, as is well known, many chemicals which affect the motor organs of man in one way or another and in some cases the effect on the motor organs is accompanied by more or less specific effects on the nervous system, including the cortex. In the hope that some light might be shed on the more fundamental operations of the spiraling mechanism in man, several walking experiments were made on two college instructors who had taken mild doses of the standardized pharmacological drug, spiritus frumenti. This drug was employed rather than caffein or theobromine or other of the commoner alkaloids of similar effect, because its physiological effects have been more widely studied and observed and also because it is generally safer to use. In addition, it has long been known to produce an exhilarating effect in the activities of the cortex of such a kind as to make the person seem temperamentally different when stimulated by it. Since, as we shall see later on, there is good ground for holding that certain types of paths are correlated with certain temperaments, this type of experiment gains added interest. The experiments were performed in a fairly level field in another part of the world where the particular experimental conditions required were generally more suitable.

The subjects R and WW walked several straightaways first to see what their normal paths are like. One of each subject is given in *R83* (pl. 9) and *WW88*. *R83* is a typical normal path for R, and but slight effect of the drug is noticed in *R84*. The spiral is more open and the drift is different in direction. *R85*, which was preceded by a second mild dose, is markedly different from the normal. There is a very long and almost straight section at the beginning of the path, followed by two circles, which in turn are followed by a long open curve. The first part of the curve is of the typical heterotropic type, although the stem is unusually long. This type of curve, as has been stated before, is associated with a near-balance between right-turning and left-turning tendencies, and this near-balance was probably brought on by the drug, for R is normally right-turning. *R86* is remarkable for the number of small circles, some of which are only 2 to 2.5 meters in diameter. All the turns are right, however. The circles are undoubtedly due to the spiraling mechanism, for the drift of the spiral away from the beginning of the path is maintained and the circles were for the most part smooth. In *R87* the circles are all small and mostly to the left (ten left to four right). The beginning of the path is the usual straight or nearly straight stretch, which indicates that the motor mechanism was still under control, although the fact that the curvature was to the left and the larger number of spiral turns were to the left, indicates that it was thrown off its right tendency into a left. In the last two paths, *R86* and *R87*, the steps were considerably shorter than in the preceding paths.

WW. The normal curve for WW is shown in *WW88* (pl. 9). After the first dose of the drug a heterotropic type of path was walked, *WW89*. This is not a typical heterotropic type of path, however, for it is too long before a circular path begins. In *WW90* the effect of the stimulant is marked. A typical heterotropic type of opening is seen and the spiral of small turns (about 16 meters' diameter) has a definite drift, showing the operation of the spiraling mechanism throughout. A second dose of the drug made the spiral very irregular, with small turns of irregular sizes, but the general drift is nevertheless away from the base. All the spiral turns are to the right and the beginning of the curve is of the heterotropic type. *WW92* is similar to the preceding except for three sharp turns which resulted from uneven topography of the field. In this path the usual nearly straight stretch of about 35 steps is present, though much shorter than under normal conditions and the heterotropic type of start is very evident. In this path, as in the preceding, all the turns are to the right, and the drift is, in general, away from the base.

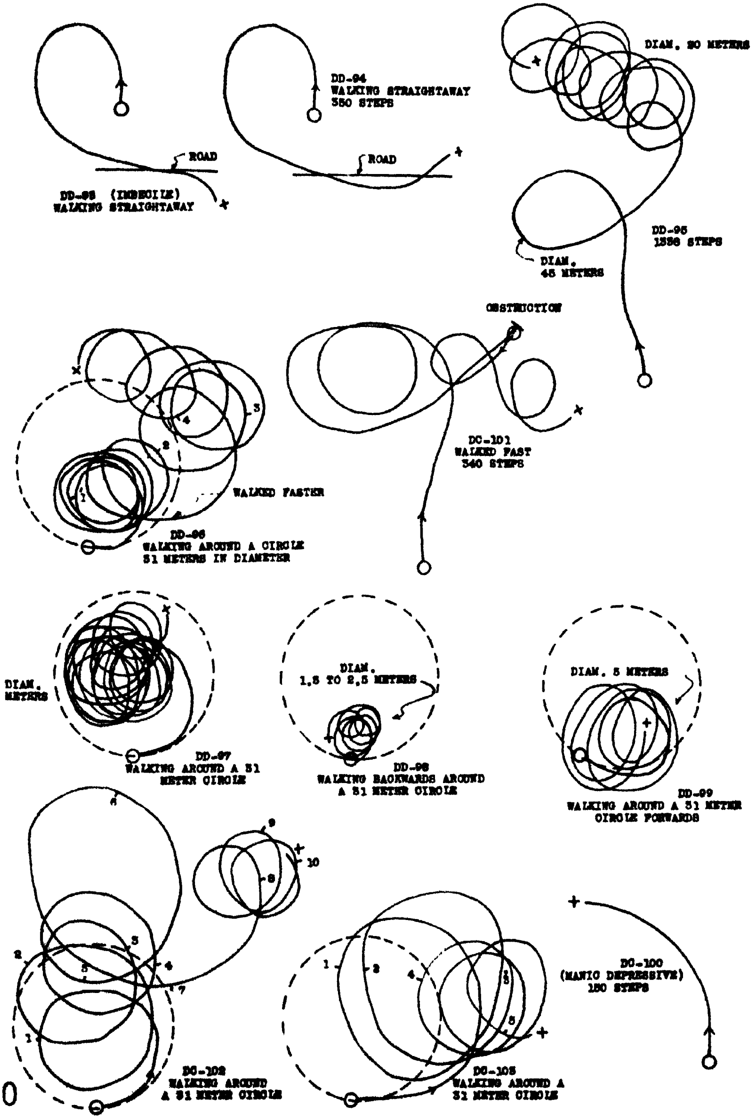
All these paths show the spiraling mechanism at work, as indicated by the beginning straight or slightly curved section; secondly, by a series of spiral turns showing a definite drift. The progressively stronger effect of the stimulating drug as the series of experiments were made caused smaller and smaller spiral turns. Just as soon as the spiraling mechanism began to operate, as judged by the appearance of the curves, in *R86*, *R87*, *WW91*, and *WW92*, it operated much more strongly, that is, made much smaller turns than under normal conditions. The drug removed some restraint, therefore, which is ordinarily present and prevents spiral turns as small as 2.5 meters in diameter. This lack of restraint is probably decreased discrimination in muscle sense.

One of the most remarkable effects of this experimental treatment is that it transformed the normal open spiral of WW into a close one as the first marked effect of the stimulant, while in R the close spiral was transformed into an open one. That is, it required a moderate dose of stimulant in each of these subjects to walk paths that resemble those the other one walks normally. No satisfactory explanation suggests itself for this peculiar circumstance, although somewhat similar results are said to occur frequently in subjective operations under similar conditions. The subjective reports from these walkers were, in general, similar to reports from normal walking experiments except that there was much greater wealth of detail. There was no sign of intoxication in the ordinary sense of that word. Such a condition would of course have defeated the object of the experiments and invalidated the results and made them wholly worthless as a contribution to the scientific study of spiral movement.

### *Spiral movement in two mental defectives*

DC. This subject is a patient in the Topeka State Hospital. He is diagnosed as a manic depressive, manic type. He is fifty-four years of age and has been in the hospital for thirty years. He works at simple tasks in a mechanical manner, speaks only when spoken to, can tell time, but says he is "one hundred years" old when asked his age. He was quite willing to walk blindfolded ('to test his eyes for glasses'), but an assistant had to follow him and constantly urge him to walk faster to keep him from stopping and trying to peep out from under the blindfold. Two of his straightaway paths (walking toward the hospital) are shown in *DC100* and *DC101* (pl. 10). *DC101* shows a typical left spiral with a heterotropic handle. After walking close to an obstruction, he was halted and led around by the assistant to the right, whereupon he made a right-hand turn followed by another left turn. A 31-meter circle was marked off on the ground and DC was asked how many steps he thought it was across. After a moment's pause he replied "about forty." He was then blindfolded and asked to walk around it a number of times until told to stop. *DC102* and *DC103* show the results.

DD. This subject is a twenty-three-year-old imbecile with a mental age of about three or four years. His hospital record states that his reason and judgment are poor, but that his sensory system appears normal, that he has no hallucinations or illusions, is well oriented as to time, place, and person, and is generally well behaved. He was quite willing to walk blindfolded, although an assistant had to follow him a part of the time to keep him from slowing down. *DD93*, *DD94*, and *DD95* (pl. 10) are three straightaway paths, the first two of which are almost exactly identical, but had to be terminated because of obstructions. *DD95* is a typical path, indistinguishable from those of normals. *DD96*, *DD97*, and *DD99* are three paths walked around a 31-meter circle. When asked how many steps it was across the circle, he replied "about six." The small circles he made indicate that he followed his judgment, for the diameters varied from 4 to 5 meters. In *DD98* he walked backward around the circle in very small circles varying from 1.5 to 2.5 meters diameter, while the smallest were only about 1.2 meters in diameter. It is clear, therefore, that in the straightaway, where judgment did not


ROAD
DD-93 (IMBECILE)
WALKING STRAIGHTAWAY
DD-94
WALKING STRAIGHTAWAY
350 STEPS
ROAD
DIAM. 20 METERS
DD-95
1356 STEPS
DIAM.
45 METERS
OBSTRUCTION
DC-101
WALKED FAST
340 STEPS
1
2
3
4
WALKED FASTER
DD-96
WALKING AROUND A CIRCLE
31 METERS IN DIAMETER
DIAM.
4 METERS
DD-97
WALKING AROUND A 31
METER CIRCLE
DIAM.
1,5 TO 2,5 METERS
DD-98
WALKING BACKWARDS AROUND
A 31 METER CIRCLE
DIAM. 5 METERS
DD-99
WALKING AROUND A 31 METER
CIRCLE FORWARDS
6
9
10
8
2
3
5
4
7
1
DC-102
WALKING AROUND
A 31 METER CIRCLE
1
2
4
3
6
DC-103
WALKING AROUND A
31 METER CIRCLE
DC-100
(MANIC DEPRESSIVE)
150 STEPS

10

Plate 10

enter into the making of the spiral turns, the size is typical of the normal spiral; but in walking around the circles where judgment entered, the paths show the effect of greatly misjudging the size of the circles.

### *Walking around figure-8's and around squares*

Two short series of walking experiments were performed on the ice of the large Wachusett Reservoir at Clinton, Massachusetts, during the winter of 1923. In one series the blindfolded subject walked around figure-8's made of two osculating circles of 9 meters' diameter scratched on the ice. In no case was it possible to approximate the figure-8 so that it could be recognized by inspection of the path merely what the object of the experiment was. As might be expected, the greatest deviations occurred where a right-hand curve changed over to a left-hand and vice versa, producing a great and irregular distortion as the subject repeated the course around the figure. In one case the direction of turn was reversed and the pattern of the figure-8 completely lost. All three subjects used in this experiment walked a number of times around one of the 9-meter circles with a fair degree of accuracy.

In the other set of experiments the object was to walk around a 9-meter square scratched on the ice and circumscribing the circle. The results were as accurate as those of walking around the 9-meter circle. All the subjects walked from 200 to 300 meters around the square without showing any curvature on the sides. The right-angle turns at intervals is evidently sufficient to prevent the spiraling mechanism from influencing the direction of the path.

In the table below is summarized the entire number of spiral turns made by thirty-seven subjects while walking, swimming, or guiding an automobile blindfolded, in attempting to go in a straight line. Other experiments, such as walking around circles, etc., are not included. The direction of the first turn of a complete spiral path is also given, as well as the number of heterotropic starts. These data probably represent a random selection which is indicated by the close agreement between the left-right ratios of the total number of turns and the initial turns. Right-turning predominates in walking and automobile driving, but left-turning predominates in swimming. (The initial turns in swimming were 24 right to 10 left.) No explanation is apparent, and it is possible that a much larger number of subjects and experiments would reverse the ratio.

TABLE 1

*Showing ratio of left to right turns in walking, swimming, and driving automobiles blindfolded*

| | *Heterotropic starts* | | *Homotropic[7] starts* | |
|---|---|---|---|---|
| | *Left* | *Right* | *Left* | *Right* |
| Direction of turn at beginning of path | 12 | 19 | 42 | 56 |
| Total starts, left-right ratio ... | 1 to 1.38 | | | |
| Number of spiral turns made in walking straightaway (20 subjects) . . . | | | 111 | 230 |
| Number of spiral turns made in swimming straightaway (14 subjects) . ...... | | | 115 | 94 |
| Number of spiral turns made in automobile driving (8 subjects) .. ... | | | 55 | 92 |
| Total .. .. ... . . .... . | | | 281 | 416 |
| Ratio .... ... ........ ... .... | | | 1 to 1.48 | |

### *Spiral movements on boats and trains and their relation to rotational movements in dizziness and vertigo*

In addition to these experiments in walking, swimming, etc., some observations were also made while riding on small motorboats, trains, and ocean liners as to the influence of the spiraling mechanism. The most definite results are obtained on motorboats and trains. When one stands facing ahead on a small motorboat going in a straight course, fixes his mind on an object in the distance and then closes his eyes, the boat soon turns off her course, either to the right or to the left. If the subject changes his position by turning on his heel so as to always face the objective in the mind's eye, he may make a complete rotation or even more on his heels, although usually the mind's eye becomes confused after half or three-quarters of a turn. The same effect is produced when facing astern, but the direction of turning is reversed in most cases, probably typically in all cases. (This can be determined only with certainty by a person who is invariably right-turning or invariably left-turning, and none such has yet made the test; such persons seem, moreover, to be very rare if they exist at all.) As a concrete illustration, one subject, whose predominant direction of turning was right when walking blindfolded, found a motorboat going to the right eleven times out of twelve trials when facing ahead. When facing astern and fixing the mind's eye on some distant object which the boat was leaving behind, the object gradually swung over to the right in three trials. This means that the boat's path was interpreted as being to the right. Similar results were obtained on three ocean liners in a fairly calm sea, but only with considerable difficulty is it possible to get the spiraling mechanism to work, and it is seldom possible to get more of a turn than about 60°. The most favorable position is the extreme stern, over the propellors. On trains the phenomenon is easily observed, especially on the observation platform. Here it is, of course, necessary that the train run on a straight course for some miles. It is also very easy to notice the operation of the spiraling mechanism in Pullman berths, but the data are of little value, since it is usually not readily ascertainable whether the train was running a straight course. But the fact that in the great majority of cases the turning is in the same direction as that observed in walking and on boats may indicate that the train was running a generally straight course while these tests were being made.

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[7] 'Homotropic' means that every part of the curve turns in one direction, either right or left, as distinguished from 'heterotropic' curves, in which some sections turn to the right, others to the left.

When one is making these tests on trains or boats and the direction of locomotion is markedly changed in a particular direction, the subject of course turns in the opposite direction to an approximately equal amount. This necessarily follows from what has been learned of the walking and automobile experiments. This is a type of compensatory movement well known in many vertebrates and in invertebrates (see especially Lyon, '00).

These observations on boats and trains are what would be expected after having seen the results of driving an automobile blindfolded. That the spiraling effect is more easily obtained on small boats and trains than on ocean-going liners is probably due to the larger amount of noise and quick movements of the small boats and trains which create a more complete mental picture of locomotion or else serve to reduce further the discriminating power of the equilibrating organs in the labyrinth than is possible on a large vessel. This hypothesis could very easily be tested experimentally, but so far no good opportunity has presented itself. That a receding objective (from the rear end of a train or stern of a boat) should turn in the same direction is, however, not so clear, for the direction of locomotion of the boat or train would in that case be to the left. As in the case of the palolo (compare p. 378), it seems that here the direction of rotation is primary and independent of the direction of progression.

When a person swims or walks a straightaway blindfolded, he is not aware of a sensation of turning as he walks (unconsciously) in spirals. There is no subjective sign of any sort that he is walking in any but a straight path. But in directing the path of an automobile blindfolded or in testing the direction of movement of a boat or train as described above, a distinct sensation of a continuous rotational 'drift' is experienced with a measured velocity of slightly less than 1.25 minutes per rotation (compare *Z58*). It is 'less,' because the body follows the drift, and one spiral turn required about 1.25 minutes. These subjective sensations of rotational drift seem to be identical with those experienced by some persons suffering from overdoses of quinine, alcohol, caffein, or other stimulants. In these conditions the rotational drifts are more intense and probably more rapid, as the experiments herein described would tend to show (*R*, *WW*, pl. 9).

Rotational drifts are also experienced in many of the large classes of nervous diseases of which vertigo or dizziness is a symptom, and this is especially the case with those diseases which are of purely neurotic origin. In the literature, the asymmetrical movements made during dizziness and vertigo and the subjective rotational drifts have generally been assumed to be due to defectively functioning semicircular canals and accessory organs, mostly because in certain diseases of the canals, especially Ménière's disease, the accompanying incoordinated movements seemed evidently directly connected with the infected or diseased canals. Likewise, in the experiments on extirpation of the labyrinth of either or both sides, pronounced incoordination of movements is produced (see especially the papers of Ewald, '92, and of Thomas, '99 and '12, who worked on frogs, salamanders, pigeons, guinea-pigs, and dogs, and the book of Maxwell, '23, for general discussion). Similar effects in some respects were also produced by extirpation of parts or the whole of the cerebellum, though in other respects the results were distinctly different, such as the ability of the operated animals to swim in the latter case while unable to do so in the former.

But there are a number of cases on record where there was no reason to suppose that the dizziness or vertigo was associated with defective canals, such as the presence of tapeworms, lowered or increased blood pressure, toxemias, etc. (Oppenheim, '11). One of the commonest elements in the neurasthenia, hysteria, etc., syndromes is vertigo, and in these cases no structural defects in the labyrinth or cerebellum seem to have been shown to exist. It is, however, quite possible that a change of blood pressure accompanying the neurasthenic condition temporarily impairs the function of the organs of equilibration, but just how, is purely speculative. For the purposes of this present paper it is, however, unprofitable to discuss all types of dizziness or vertigo, for many of them are undoubtedly associated closely with structural pathological defects in the labyrinth or cerebellum; and in many of the remainder of the cases the observational data of the case histories are not complete enough to show whether rotational movements or drifts were experienced. For at this stage of the investigation, the only definite connection between spiral movement and vertigo consists of those cases where a subjective rotational drift was reported. (This is to be taken in a practical sense, of course, and not in a theoretical; for when the labyrinth ceases to function through disease or removal, the resultant dizziness or vertigo is not caused by something which is no longer there, but by something which is still there, but which functions differently from the equilibrating organs. It is absurd to say that the extirpation of the labyrinth of the frog causes inability to swim, for example. The special character of swimming is plainly produced by something which is still in the frog.)

If the end organs of the eighth nerve cannot be shown to cause the rotational movements and possibly resultant dizziness or vertigo, where, then, is the seat of these phenomena? The hypothesis adopted in this paper is that it is in the organization of the nerve tissue itself, that it is a temporarily accentuated property which is present in normal conditions in all animals as exhibited by the phenomenon of spiral movement. Loeb ('91), on dogfish; Ewald ('92), on frogs; Laudenbach ('99), on the amphibian, Siredon; Trendelenburg and Kühn ('08), on snakes and lizards; Thomas ('12) and Wilson and Pike ('12), on dogs, showed that with both of the eighth nerves severed, these animals rotated on the long axis when swimming—a result which obviously cannot be explained by the absence of the labyrinth nor on the basis of bodily asymmetry (compare pp. 354–361 of this paper).

Lee ('94) obtained somewhat similar results from his work upon the dogfish, and Mr. David Pankratz and myself obtained confirmatory results on the toadfish (Opsanus tau) in two specimens which had the eighth nerves severed and the eyes removed. Practically all the swimming was in helical spirals with the turns wound closely together. One of the fish swam the turns so closely together at times that they would ordinarily be described as circus movements, although there was definite but slight forward movement along the spiral axis. Streeter ('06) also performed some very interesting experiments in this connection. He removed the ear vesicles in very young frog embryos and kept them alive for about two months, when they died, owing to general inability to go to the surface of the water to get air and to find food. These tadpoles sometimes swam in spirals.

In this connection it is necessary to insert a note on 'circus movements' and 'forced movements,' which result when one of a pair of sense organs employed in equilibrium, such as the eyes or the labyrinth, is removed or otherwise prevented from functioning. That the functioning member of a pair of sense organs under these conditions exerts a powerful influence on the behavior because of the removal of the opposing sense organ, and thereby destroying the 'balance' of the equilibrating mechanism, seems to be generally admitted. The circus movements such as insects make, for example, when one eye is blackened, are not caused by the spiraling mechanism, nor does it appear that the spiraling mechanism is a component in causing the resulting behavior. This is shown most clearly by the fact that the removal of one orienting or equilibrating organ is associated with a change of behavior referable to the side of the animal operated on, while the right or left turning in movement due to the spiraling mechanism is independent of all discoverable bilateral asymmetries. Then again, animals with unilateral lesions frequently recover gradually from their asymmetrical behavior, while there seems to be no 'recovery' from the operation of the spiraling mechanism when orienting senses are not functioning. Since circus movements are therefore not directly related to spiral movement, further discussion of them is not relevant here.

It seems, therefore, that when the ordinary functioning of the equilibrating organs is interfered with through some psychic, vasomotor, or other disturbance, or completely obliterated by experimental means, the more deep-seated spiraling mechanism makes itself felt and produces movement in a helical spiral of a greater or less degree of regularity, depending upon vigor of the locomotor organs and other conditions.

As has already been said, the spiraling mechanism makes no report in consciousness when walking or swimming or driving an automobile blindfolded. But when one is directing an automobile by calling directions to the driver or when testing the straightaway path of a boat, one becomes aware of a rotational drift. It is evident, therefore, that the spiraling mechanism may break through into consciousness in some way when it moves at a more rapid rate, so to speak, than the body does. Under abnormal psychical conditions, this rotational drift becomes sufficiently pronounced to become of pathological importance. Whether the nature of such abnormal psychical conditions is, at bottom, reduced discrimination of the end organ of the eighth nerve, is not definitely known, but we may have an earnest in the fact that in automobiles and small boats, with the increased amount of noise and vibration, the spiraling mechanism works much more quickly and efficiently than on an ocean steamer; and on a steamer the spiraling mechanism works more effectively at the stern, amid the noise of the propellers, than in the staterooms or other more quiet parts of the deck.

Neither is it necessary that the rotation should be continuous in one direction or at uniform speed. The experimental records described in this paper show a great variety of form of path, and there appears no reason why, under the abnormal psychic or other abnormal bodily condition, the spiraling mechanism should not function with somewhere near the same degree of regularity.

There is a class of phenomena closely related to those just considered, so closely related, in fact, that they should be considered as of the same class. These phenomena are usually called compensatory movements. They are seen not only in vertebrates, but in many kinds of invertebrates. In fact, Lyon ('00) reports that all types of compensatory movements known in vertebrates can be demonstrated in insects where no otoliths and, of course, no semicircular canals occur. In crabs and crayfishes the compensatory movements are in large part due to the presence of an otocyst and a small part to sight. But a small part is also due to some other influence besides sight and the otolith. In insects the compensation seems completely or almost completely mediated by sight.

But dependence on sight for compensatory motions and maintenance of equilibrium is very likely also the case in some human deaf-mutes. James ('82) investigated 519 deaf-mutes and found that there were a considerable proportion who did not become dizzy on being experimentally rotated, as is now being done in the Barany tests for nystagmus and 'past pointing,' which indicates that their internal ears were not functioning. Many of these deaf-mutes were unable to swim, not knowing up from down, nor any other direction, when immersed with the eyes closed. Many of them also had great difficulty in walking in the dark. It is almost certain, therefore, that in these non-dizzy deaf-mutes the function of equilibration was for the most part taken over by sight. This is confirmed by Wilson and Pike's ('12) labyrinthless dogs which became practically helpless when blindfolded, although when seeing they got along fairly well.

In looking at all the observations, therefore, experimental and otherwise, on equilibration and compensation movements in vertebrates and invertebrates, the following statements would seem to apply: It is more difficult to maintain equilibrium in water than in air for vertebrates and invertebrates alike, which follows presumably from the smaller difference in specific gravity between the organism and the medium in the two cases. Special organs may therefore have arisen in crustaceans, viz., the otoliths to subserve this function, while the insects found sight capable of mediating equilibrium. In no organism is equilibrium maintained exclusively by the internal ear or by the otoliths; sight, the muscle sense, and perhaps also other senses also play a part. When the special equilibrating organs are thrown out of function for some reason, other senses, such as sight, may take it over as in the non-dizzy deaf-mutes. Because of the varying sensitiveness of the different sense organs of different individuals, the part each sense organ has in maintaining equilibrium varies; and since other sense organs can take over in large part the function of maintaining balance, observation on extirpation experiments must of necessity vary considerably and progressively as the animal lives on and readjustments set in. Out of this fact arise undoubtedly many of the supposedly discordant results in the literature on this subject.

What we know, therefore, of the functions of the cerebellum and the labyrinth by experiment on various animals and from clinical observation on man, together with what we know of the spiraling mechanism in man, indicates that a considerable proportion of the cases of dizziness and vertigo find the immediate cause in the operation of the spiraling mechanism through the subsidence of functioning of the labyrinth due to pathological or abnormal psychic conditions.

### *Note on the autokinetic sensation*

Closely related to the observations on rotational movements on boats and trains, etc., are those comprised under the so-called autokinetic sensation, to which my attention was recently directed by Dr. J. P. Guilford, who is engaged in investigating this interesting phenomenon. The literature on this subject is rather extensive and dates back over one hundred years to an observation by von Humboldt. For a review of the principal papers reference may be made to the papers by Doctor Guilford, now in press in the Journal of Experimental Psychology.

A good illustration of the autokinetic sensation is had when one gazes intently at a lighted cigarette in a dark room. Under these conditions the light spot of the cigarette begins to move. The path it describes when the gaze is held fixed varies somewhat with the subject, but most frequently it is some form of oval, and the rate with which it moves is in the same range as that observed in walking, swimming, or driving a spiral turn or to rotate on the heel in maintaining a constant relation to a position fixed in the mind's eye on a moving boat or train; that is, from 1 to 2 minutes for a turn, with extremes of ⅓ minute and 2.5 minutes.

In order, however, to make the conditions of these experiments more nearly comparable with those of the walking experiments, I attached the lighted cigarette to the hat brim by means of wires to eliminate vibration, about a meter from the eyes. When one now fixates the eyes on the dim spot of light, it begins to move after a few seconds just as it does when lying on a solid support. With care one can follow this apparent movement by rotating on the heel so as to make one or more complete rotations on the body axis, and the time required for one rotation is found to be within the same time range as that noted for walking, swimming, or driving circles. It is to be noted that one must follow the 'movement,' that is, go a little slower, or the illusion will be destroyed, and one has to begin all over again. Many of the details of observation surrounding these experiments on the autokinetic sensation, as well as those recorded in the literature, are so similar to those observed in the experiments on boats and trains and automobiles, that there can be little question that the spiraling mechanism causes the dim spot of light to move, and that the autokinetic sensation is, in fact, a special case of spiral movement.

It is indeed probable that every sense organ that has to do with localizing a point in space is subject to the influence of the spiral mechanism, so that when the rest of the space field does not contain definite reference points or landmarks which tie the field together, the point fixated will begin to rotate. With appropriately devised experiments, both hearing and touch could doubtless be thus investigated.

## DISCUSSION

It is the object of this section to make a critical analysis of the experimental data set forth in the preceding section in order to see what conclusions may be drawn from them and what connection there is between these conclusions and those derived from a study of movement and certain aspects of the structure of other organisms. As stated in the introduction, these experiments were carried out as a part of a more comprehensive study of spiral movement in organisms. The belief was held from the beginning that no one group of organisms would probably suffice, by using the experimental method, to explain the fundamental characteristics of the spiral mechanism, but that some phases at least of the workings of this mechanism could be much more readily understood by examining such organisms as might be supposed to be more suitable for a particular purpose. Thus, for example, where could one find an organism better adapted than man to test the effects of changing the relation of the body axes to the position of the path? From this larger point of view, therefore, man is neither more nor less important than the ameba, for example, in shedding light on this subject; and it is entirely as reasonable to study man's movements in order to understand how an ameba moves as the other way around. The most efficient organism for this purpose is not the one that stands highest in the evolutionary scale, for, as will be seen, there is comparatively little, if any, evolution in this mechanism; nor is it the one that excites the most human interest on the side, but the one that reveals most of the spiraling mechanism. It is important that this is thoroughly realized, especially by those who are primarily interested in human behavior, for there is already enough evidence on hand to make it highly probable that the same mechanism is at work in man that operates in the ameba. And those anatomical structures of brain and muscle and sense organ which are peculiar to mammals or vertebrates cannot offhand be assumed to control a mechanism which functions perfectly in animals lacking these structures (e.g., Lyon's ('00) observations).[8] This study of spiral movement presupposes, therefore, that the locomotor system of all other motile organisms is before the mind when that of man is specifically under discussion.

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[8] Lyon, for example, found compensatory movements in a number of invertebrates where semicircular canals do not, of course, exist.

The spiral movements of man, however, do not stand forth as a closed system of behavior; these movements, on the contrary, are more or less influenced in their expression by other activities of the organism. As to how extensive and varied these influences are, these experiments afford only a preliminary glimpse. So far they seem mostly to be concerned with matters of detail (leaving out of account now hereditary differences of brain structure, involving 'temperament') which in no case so far obliterates, even to an appreciable extent, the general effect of the spiraling mechanism. With these details this study has little to do, except in so far as a study of them may lead to a further understanding of the spiral mechanism. When a feature of movement is seen to be due to some stimulus that bears no definite relation to the study of spiral movement, it is not studied further. These extraneous features are stripped off, however interesting they may be from other points of view, as of no further concern in the present investigation.

In comparing these experimental data with each other and with similar observations from other animals, we may consider, however, for special examination the supposed connection between structural asymmetry and walking around in circles when lost, because this erroneous belief is so widespread and because certain writers have actually measured skeletons of men and other vertebrates in an effort to discover thereby such connection. So that, although it seems impossible to maintain further that asymmetry is the cause of spiraling, from even a superficial examination of the foregoing experiments, it may be necessary, in order to satisfy all concerned, to go further into the details involved in this question and to examine every important detail of the asymmetry hypothesis. For it will be seen that, although the spirals are not due to asymmetry as stated, certain details of some paths may be caused by asymmetries.

In so far as the recorded observations go in this paper, What is the evidence against explaining the spiral path as due to asymmetry, such as a longer leg on one side, for example, or stronger (heavier) muscles in one leg than in the other?

1. In the first place, structural asymmetries are permanent. A longer femur remains a longer femur and a heavier set of muscles in one leg than in the other remains heavier. And the result in the functioning of such asymmetrical legs, other things being equal, is that the longer or stronger one takes longer steps and thus the body is led to go in a circle or in a series of circles. Observation shows, however, that both right and left circles are frequently walked or swum in the same experiment. If there is structural asymmetry of the kind specified above in this person of both right and left turns, some factor operates which is just twice as powerful as the effect of the asymmetry, either when the first circles are made or when the second (opposite) set is made. And the factor has exactly the effect of the asymmetry, except that it is opposite in effect, since the circles are just as smooth and of about the same size.

Or one may assume that in this case there is no asymmetry, both sides of the body being considered equal in size of bone structure and strength of musculature. But since one observes the same shape and size of circles in these experiments as in others where the spiral turns are all in one direction, the assumption of asymmetry to explain spiral turns is no more necessary in the latter homotropic spiral than in the former.

As we shall see later, the degree of asymmetry reported from measurements of skeletons differs from a very slight to a marked degree. This would lead one to expect a great variation in the size of the spiral turns among different individuals, those with marked asymmetries walking very small circles, while those with very slight asymmetry should walk very large circles, and those with no asymmetry should walk practically straight lines. And, again, the size of the spiral turns should remain fairly constant for the same individual. As against these theoretical expectations, the experiments show that there is indeed considerable variation in the size of the spiral turns among different individuals, but the variation is not regular for the particular individuals; that is, the spiral turns vary in size considerably in the paths of any given individual, the first few turns being frequently several times the diameter of the later turns. None of the thirty-four subjects tested has been able to swim or walk a reasonably straight path, except a very short one.

2. How could asymmetry of the bones and muscles between the two sides of the body explain the spiral paths of an automobile run by a blindfolded driver? Certainly, no asymmetry below the shoulders could account for the spiral path. And even if it is assumed that the muscular asymmetry between the arms is effective, how would one explain the fact that some paths are left and others are right, while all the recorded paths were driven by right-handed persons? The automobile paths made by calling the directions to the driver seem quite impossible to explain on the asymmetry hypothesis, seeing that they are in all essential particulars similar to walking and swimming paths.

3. If asymmetry is at the bottom of spiral movement, one would expect that a change of swimming stroke would show a change in the curvature of the path, yet this does not always happen. It seldom happens, in fact, unless the change is from a smooth to an awkward stroke, and in these cases the change in the curvature of the path is often only slight or delayed for a considerable time after the change in stroke was made.

4. Perhaps the strongest simple evidence against the asymmetry hypothesis consists of those experiments in which forward and backward walking straightaway were alternated regularly. Undoubtedly, this test should remove extraneous influences because of the frequent changes of direction and allow asymmetries to express themselves functionally. But some of these paths are as regularly spiral as any that are walked continually forward. But in one or two of these curves there are sinusoid handles which may possibly represent some asymmetry, for the forward sections veered to

the right while the backward veered to the left, in path *Z56*, for three forward and three backward sections. The third backward section was nearly straight, indicating an almost complete mastery of the right spiraling mechanism, which won complete mastery in the following forward section and then maintained it to the end of the path. In view of the marked difference in curvature between the forward and the backward sections in the beginning of the path and the smoothness of the spiral turns in the latter section of the path, where the forward and backward sections are harmonized into a curve indistinguishable in smoothness of arc from a forward path, one can get a very good idea of the power which the spiraling mechanism exerts in bending the neuro-muscular mechanism to its will.

5. The comparative straightening out of later sections of the path, due to walking circles by design in the first part of the path, cannot, of course, be regulated by a rigid mechanism such as is subsumed under the asymmetry hypothesis. Not only is the path straightened out, but in a few cases a reverse circular turn is made, and these reversed turns may be in a direction opposite to the spiral turns usually made by the particular individual when walking a straightaway blindfolded.

6. Finally, there is the interesting fact that there is no definite correlation between the known asymmetry of left-handedness or right-handedness and the preponderant direction of the spiral turns. In the first place, many of the right-handed subjects walked or swam or drove an automobile in spirals in some experiments to the right and in others to the left. In the second place, only left turns are on record for some of the right-handed persons. Third, of the three left-handed persons, two walked right-hand turns in one experiment and left-hand turns in another, and the other walked all right spirals in all the straightaway experiments.

### *What is known about structural asymmetry?*

The best-known asymmetry in man is undoubtedly the greater strength of the right arm as compared with the left in the great majority of persons. This asymmetry in the use of the arms is, of course, directly dependent on the greater development of the muscles, but the discovery of this correlation was probably made much later. So far as the records go, right-handedness (or left-handedness) was noted very early. Perhaps the earliest record which seems to give some idea of the dominance of right-handedness is the case of the 700 Benjamite soldiers who were picked out from among 26,700 soldiers 'for their superior skill' (The Bible, Judges, iii, 15; xx, 15, 16). This seems to indicate that there were at least 2.6 per cent left-handed among the soldiers. Hasse and Dehner ('93) found 1.14 per cent left-handed soldiers in a total of 5141. No ambidextrous individuals are reported.

But the first actual measurements of structural asymmetry were made many centuries after the functional asymmetries were discovered and recorded. These first measurements were made by the anatomist F. Arnold in 1844. He found that the right humerus in fourteen out of sixteen skeletons was longer than the left; and the right forearm in twelve out of sixteen was longer than the left. On one case both arms were of the same length and in one case the left arm was longer. Arnold made another contribution. He directed attention to asymmetry in the legs where he found the right femur longer than the left in four out of the same sixteen skeletons; the left femur longer than the right in eight out of sixteen skeletons, and equality of length in four cases. He thus established the prevalence of what has since been called 'crossed symmetry,' that is, that left-footedness frequently goes with right-handedness. He also claimed to have found perfect equality in length in the bones of the right and left sides of the skeletons of twenty fetuses ranging from three to ten months. This idea held sway until the present time, when more precise means of measurement and more refined technique showed that asymmetries are present to about the same extent in fetuses as in adults (Schultz, '23, '26; Bartelmez and Evans, '26). Following is a table by Schultz ('26), showing asymmetry in the length of the humerus and in the length of the foot.

TABLE 2

*From Schultz, showing asymmetry in humerus and foot length in fetuses and adults*

| | | | *Right greater than left* *Per cent* | *Right=left* *Per cent* | *Right less than left* *Per cent* | *Average per cent difference* |
|---|---|---|---|---|---|---|
| Humerus length | Fetal | 100 | 52 | 27 | 21 | 1.3 |
| | Adult | 105 | 54 | 24 | 22 | 1.5 |
| Foot length | Fetal | 100 | 33 | 20 | 47 | 1.4 |
| | Adult | 500 | 31 | 16 | 53 | 1.1 |

Weber ('49) concluded from his studies of asymmetry that the symmetry of body build stands in special relation to locomotion (left-handedness, etc.). Manouvrier ('82) measured the skeletons of six gorillas and found the right femur heavier in two cases, the left heavier in three cases, and no difference between the right and left in one case (range 1 to 39 grams). Gaup ('89) measured asymmetries of a similar kind in a large number of birds and mammals (jaguar, panther, raccoon, etc.), but no systematic difference could be detected in the length of the bones of their extremities.

Later on, careful measurements by Guldberg ('96) on skeletons and living men confirmed Arnold's measurements. Guldberg found the right arm predominant in about 75 per cent of the cases, the left in about 8 per cent, and equality in about 15 per cent, in both skeletal and living material. And in the legs, right predominated in about 23 per cent, left in 51 per cent, and equality in 26 per cent. Hasse and Dehner ('93) measured a much larger number of men (5141 soldiers) and reported the right arm longer in 75 per cent, the left in 9 per cent, and equality in 16 per cent. They found the left leg longer in 52 per cent, the right in 16 per cent, and equality in 32 per cent. These authors also made dynamometer tests on 190 recruits, finding the right arm stronger in 72 per cent, the left in 18.4 per cent, and equality in 8.4 per cent. In another set of twenty-one men the percentages were, respectively, 80, 10, and 10, and the right leg was stronger in 38 per cent, the left in 52.4 per cent, and equality in 9.5 per cent.

No studies showing correlation between length of bones and strength in arms and legs seem to have been made. One can therefore only use statistical averages of the various measurements, which may, however, be presumed to show approximately the actual correlation still remaining to be directly measured. The general results of the measurements of recent date indicates that from 1 to 2 per cent are left-handed and about 98 per cent right-handed, while the left arm is longer in 9 per cent and the right in 75 per cent. That is, the left-handed group is much smaller than would be expected from structural measurements. It seems that all the persons having arms of equal length and 80 per cent of those having the left arms longer have in some way become merged with the 'right-handed' group. It is quite probable that much of this transfer is due to early training in school and at home. Right-handedness is the mode and many mildly left-handed children undoubtedly become transformed into 'right-handed' ones through training.

Curiously enough, we have almost no data on right-footedness and left-footedness. No respectable scientific reason can be assigned for this absurd neglect, seeing that the arms have received so much attention. From such meager data as we do have, it seems that the amount of functional left-footedness corresponds approximately with structural left-footedness. That is, in over 5000 men the left leg was found to be longer in 52 per cent and in 21 recruits the left leg was found stronger in 52.4 per cent. There is therefore a 'crossed symmetry,' both structurally and functionally, in the majority of cases; but here again the condition on an individual basis is not known, except in a very small number of cases. It can nevertheless be concluded that there must exist an actual correlation between strength and length of legs and arms, but of what value this correlation is, still remains to be determined.

From still another point of view, it is difficult to see how the existence of crossed-symmetry should be so consistently overlooked, for the common vertebrates, horses, cows, dogs, etc., show highly developed crossed functional symmetry in the legs when walking and running. That is, the front right leg and the hind left leg are moved together and alternately with the other two legs. In pacing we have, of course, an exception: is the symmetry lateral here? The same is true of monkeys in climbing up the sides of the cage and of creeping babies. This phenomenon is so widespread and appears so early in the life of the individual vertebrate that it seems there must be a morphological basis for it. It is astonishing that so far as I have been able to find, this phenomenon has been wholly overlooked or ignored in discussions of left-handedness or right-handedness.

With respect to the present study of spiral movement, asymmetry of arms, whether structural or functional, has probably little to do directly, except in swimming. But asymmetry in the legs would naturally call for careful examination. Taking the dynamometer results above quoted, it is seen at once, as Hasse and Dehner pointed out, that asymmetry, both structurally and functionally in the arms is independent of asymmetry in the legs. It is curious, therefore, that Remaley ('13) should have come to the conclusion that "left-handedness may be attributed to abnormal development of the right cerebral hemisphere" (Just what does 'development' mean?), for left-handedness may be associated with right-footedness in 40 per cent of the cases and with left-footedness in 60 per cent.

Guldberg ('96, '97), like Weber, thought that symmetry in locomotion depended upon symmetry of the body. He ('97) measured twenty skeletons of various races with results similar to those recorded above. But, in addition, he weighed the muscles of the legs of three young dogs, which he had observed to swim in circles after blindfolding. One of the dogs was two months old, the other two were four months old. They swam in circles from 2 to 5 meters in diameter (table 3).

These observations were thought to bear directly on the underlying cause of spiral movement, and, so far as they go, these three cases show a correlation between asymmetrical musculature and direction of turning. The correlation in the second dog is somewhat doubtful. To assume, however, that swimming in right circles is due to heavier muscles on the left side is hardly justifiable on the basis of three observations when unsupported by any other data. In the first place, it is not recorded how many circles were made in swimming nor whether sufficient time was allowed to change direction, as often happens in men and in mice (Dunkelberger, '26).

TABLE 3

*From Guldberg, showing comparative weights of muscles of the right and left legs of three young dogs*

| | MUSCLES OF FORE LEG | | | MUSCLES OF HIND LEG | | |
|---|---|---|---|---|---|---|
| | Weight *Grams* | Preponderance | Per cent | Weight *Grams* | Preponderance | Per cent |
| 2-months dog swam left circles | 20 | Left, 0.1 | .05 | 210 | Left, 1.68 | .8 |
| 4-months dog (a) swam right circles | 330 | Right, 2.68 | .8 | 540 | Left, .27 | .05 |
| 4-months dog (b) swam right circles | 376 | Right, 6.4 | 1.7 | 480 | Right, 7.2 | 1.5 |

Secondly, it is still open to question that muscles 1 or 2 per cent heavier in the left leg, for example, are more effective in causing circular swimming than slightly longer right legs or slightly greater spread away from the sagittal plane of the right legs. Nor is it recorded whether the body of the dog was bent in the direction of the circular path. These criticisms can be urged against making general conclusions on spiral movement in dogs as arising out of muscular asymmetries such as Guldberg found, for the behavior of mice under similar experimental conditions goes against such generalizations, as Miss Dunkelberger's large number of tests shows. And there seems to be no good reason for assuming that dogs and mice are fundamentally different in this respect.

With only one exception all of Dunkelberger's mice swam both right and left spirals, with varying degrees of preponderance to the right or to the left, but yet not with that degree of exclusiveness which would seem to be expected if the direction of turning were directly correlated with muscular or other gross structural asymmetry, as Guldberg suggested.

These investigations on structural and functional asymmetry may be briefly brought together as follows, in so far as they bear on spiral movement: 95 to 99 per cent of random groups report themselves as right-handed, while dynamometer tests show approximately 75 per cent to have stronger right arms and about 75 per cent have longer right arms. As to the legs, about 52 per cent have longer left legs and about the same percentage have stronger left legs. Right-handedness does not mean a stronger or a longer right arm; it may mean that in most cases, but in a considerable number it means that the right hand is trained to do a larger number of muscular acts requiring good coordination, such as writing, than the left. Slightly less than 25 per cent of all persons could, presumably, be trained to use the left hand as well as the right or somewhat better, for those muscular acts which they ordinarily do with the right. It is greatly to be regretted that there are no data on right-footedness and left-footedness for the sake of comparison, to see whether or not the proportion of functional right-footedness and left-footedness varies as much from the structural, as is the case, mutatis mutandis, with the arms. The leg-length asymmetry seems to vary about as much as the foot-length.[9]

Now, taking the right-handedness proportions, the dynamometer results, and the length measurements, what correlation is there between any of these and the direction of spiraling in man? The length measurements show a considerable proportion of right and left arms or legs as equal (about 25 per cent). According to the asymmetry hypothesis, persons with symmetrical length of legs (other things being equal) should walk a fairly straight path. No person, however, has so far been found to walk any but a spiral path of a greater or less number of turns. Again, there should be a preponderance in walking, of turning to the right because of stronger left legs in about 52 per cent of all persons. No such preponderance is actually found. Out of 20 persons, 8 preponderated in turning to the left, and in swimming, 8 out of 12 swam to the left. The swimming ratio approaches somewhat to the ratio of length and strength asymmetry in the arms, if it is assumed that the legs play little part in directing a blindfolded swimmer. This rough correlation between the percentage of persons with longer right arms and the number swimming to the left as recorded in these experiments, is the only correlation detectable on the basis of known asymmetry, and it is a surface correlation only. For, since 5 of these swimmers who predominantly swam to the left also swam one or more times to the right, it must be assumed that there is another mechanism present that is at least at times, more effective than the assumed permanent structural asymmetry under discussion. From present knowledge, therefore, the asymmetry hypothesis fails to explain left-hand preponderance in swimming as it fails in explaining spiral movement in all other cases in which it has been held to apply.

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[9] These structural measurements and dynamometer tests, it seems, should be taken into consideration in the genetical treatment of the inheritance of right-handedness rather than to rely on the popular notion of 'right-handedness' and 'left-handedness,' which is a wholly different thing.

Before leaving this subject of asymmetry, it may be well to call attention to the ridiculously perfect symmetry of function and structure in all other parts of the legs and arms and body which the asymmetry hypothesis tacitly assumes exists apart from the particular structure, for example, the femur, which may be under discussion. A femur longer by 5 mm. may make a leg more 'efficient' for walking purposes, but this advantage may be offset or reinforced by many other asymmetries, such as a longer or shorter foot, longer or shorter calcaneum, variation in the position of the hip-joint, in the insertion of various muscles, in their innervation, etc. With asymmetries known to occur in a great many of the symmetrically placed structures, it would, on the face of it, seem exceedingly improbable that any single asymmetry would explain the behavior in spiral movement. Only the demonstration of a very high degree of correlation between the occurrence of asymmetry and a special feature of spiral movement would entitle the asymmetry hypothesis to serious consideration. No correlation of this kind has so far been shown to exist.

### *The amount of functional asymmetry necessary to produce spiral movement*

It is obvious that a person's muscular mechanism must function asymmetrically in order to walk around in circles and in spirals. It is extremely difficult to get direct quantitative data on the amount of asymmetry of function, for walking and swimming are very complex acts and there is considerable variation in the length and swerve of the steps in walking blindfolded, although the total result often is a series of regular spiral turns. That is to say, the directing mechanism averages off the deviations caused by slight unevenness of the ground, etc., instead of following up such deviations as a machine might do. The legs do not walk the man: the man walks the legs.

There seem to be only two ways in which the legs can act in order to walk around a circle; one is by taking shorter steps with one leg with the feet kept well apart, and the other is by swinging the legs, or one of them, to the same side instead of straight ahead. The latter kind of walk will produce a circle if the steps of both legs are equal, only if both feet walk on a line corresponding with the circumference of a circle. From actual observation of short stretches of path the steps are seen to be of uneven length, of varying amounts of swerve and of varying distances, laterally, between the feet. The average amount of swerve or of longer steps on the outside of a circle or turn of a spiral can be computed, however, from measured paths, if certain assumptions are made. These assumptions are that the spiral turns are due to a definite extent of either swerve or longer steps with one leg, while maintaining a definite distance between the tracks made by the two feet.

Now, assuming that in walking a circle of 30.7 meters' diameter the curvature of the path is due to swerve alone, then each step of 760 mm. must swerve on the average 20 mm. That is, in walking a left circle, each foot must hit the ground 20 mm. to the left of straight ahead. To walk a 61.4-meter circle the swerve is only 9 mm. to each foot. For a 6.3-meter circle (*C5*) the swerve is 90 mm. but if the curvature of the path is due to longer steps with the leg on the inside, toward the center of the circle, then for a 60.4-meter circle the steps would have to be longer on the outside by 2.5 mm., if the feet from center to center are 10 cm. apart. For a 30.7-meter circle the steps would have to be longer by 5 mm., and for a 6.3-meter circle, 13 mm.

The process of taking longer steps and of swerving to the side are separated here merely for the purpose of analyzing the only, or at least the important, factors concerned, and of examining them in the light of actual observations.

In walking circles by design, blindfolded, the walker is not able to keep on the track with any degree of accuracy if the diameter is more than about 30 meters. Twice this diameter is enough to throw everyone off the first or second time around. Circles smaller than 30 meters are usually easily reproduced more or less well in the path. And the spiral turns in straight-aways, as has been shown, vary from about 5 meters to about 30 meters. Are these turns due to taking regularly longer steps with the outside leg or are they due to lateral swerving of the legs or body?

This question is important, but it is very difficult to get accurate data on the character of the steps in an actual path for lack of facilities and recording apparatus. But much of the difficulty can be disposed of in another way. The exact point at issue is: Can the presence of a mechanism be demonstrated in the body which enables a blindfolded person to increase the length of his steps regularly with one of his legs by 5 mm. to 12 mm., such as is presumed by the asymmetry hypothesis operates in walking circles? To test this in a preliminary way, six persons were asked to walk short stretches in a long hallway at an easy gait. The soles of the shoes were dusted with chalk to record the steps accurately. The steps were then measured carefully, both as ordinary steps and as Roman paces (the distance between two successive footprints of the same foot). It may be said parenthetically that the Roman pace expresses a true physiological concept in locomotion and is preferable in scientific investigations in walking to the 'step' which introduces a variable, since the step is approximately one-half of a Roman pace, bisected by the foot on the opposite side. The results of the measurements showed that there is a comparatively large amount of irregularity in the length of steps ranging from 3 mm. to 50 mm. in ordinary steps. This is under the most favorable conditions, that is, on a level floor and with the eyes open. Out in the field, blindfolded, the variation is much greater, due to the uneven surface, as can readily be observed.

It should also be mentioned in this connection that actual swerving of the body is often observed when blindfolded persons walk in small circles, but the exact amount cannot, of course, be stated, for there are no instruments available to measure it. But there can be little question of the much greater importance of swerve of the body giving swerve to the legs in walking circles as compared with asymmetrical length of steps and the presence of the ability of much finer (linear quantitative) discrimination in the swerving mechanism than in the step-lengthening mechanism. The great irregularity which is prevalent in the length of the steps therefore precludes the explanation that walking around in circles or spirals is due consciously or unconsciously to changing the lengths of the steps of one leg.

### *The significance of the drift of the spiral turns*

It is observed in the paths presented in the preceding section on experimental results that, whenever there were a number of regular turns in a path, these turns nearly always drifted in the same direction as if they were turns of a helical spiral projected on a plane surface. The general direction of this drift is away from the starting-point, and in a majority of cases approximately at right angles to the handle of the path and in the same direction as the first spiral turn. That is, if the spiral turns went to the right, the drift of the spiral turns was to the right and generally perpendicular to the handle of the path. In only a few cases was the drift in a direction opposite to the curvature of the handle of the path, and in only one case (*T41,* an automobile path) did the series of spiral turns turn back over the starting-point of the path.

There must, therefore, be some significance in the direction of the drift of the spiral turns, which seems, typically, to be in a lateral direction with respect to the handle of the path, and in the same direction in which the spiral turns are made. This condition is presumed to be typical because the largest number of spiral paths fall in this class and a much larger number of spiral paths with a large number of spiral turns fall in this class than in any other. If the direction of the drift were a mere chance phenomenon, the paths should, of course, distribute themselves more or less equally around the points of the compass. The other significant feature about the drift of the spiral turns is the uniformity with which they are spaced from each other in the larger series of turns. There may be variation, however, in the degree of such uniformity, such as might be caused, for example, by a growing sense of fatigue, but under ordinary conditions the spacing in the longer series is remarkably uniform and indicates the operation of a definite factor or a definite phase of the spiraling mechanism.

Just exactly how is the drift produced? So far as the graphic representation is concerned, the drift is produced by a rhythmic decrease of convexity at the completion of each spiral turn. That is, the line connecting any two spiral turns is less convex than the rest of the spiral turn (see especially

*Z56*). When the amount of decrease of convexity is uniform and recurs at each spiral turn at the same corresponding point in the periphery of the circle, the projection of a uniform helical spiral is the result. This is of great interest since it means that the spiraling mechanism works rhythmically in the degree to which the body swerves and perhaps also to some extent in rate of movement. That the swerve is the principal if not the only cause is made clear, however, by the spiral automobile tracks, where the speed was not under the control of the person steering. In other tracks there was perceptible, however, a rhythmical difference in the speed of walking. In *R87* the small spiral turns were walked with markedly greater speed than the straightened sections between the turns. And some of Miss Dunkelberger's mice tracks, some of which I observed while they were being made, also consisted of small turns with longer straight stretches between, and here the mice also swam considerably faster around the spiral turns than in the straight stretches. But running paths, when compared with walking paths, show larger spiral turns in the running than in the walking paths. A rhythmic burst of speed might therefore be expected to decrease the convexity of the path at the corresponding points. But in the mice tracks and in *R87* and tracks made under similar conditions just the opposite correlation is found. Moreover, in the *R87* type of track, the bursts of greater speed are not of conscious origin, while the running paths are, of course, consciously so performed. It seems clear, therefore, that the rhythmic bursts of speed in the mice tracks mentioned and in *R87* are due to some factor directly bound up with the spiraling mechanism.

In tracks of the type of *Z56*, which resemble the *R87* type in the matter of small spiral turns with comparatively flat curves connecting them, no greater speed was observed in walking the spiral turns than in the connecting stretches, but a slight or even a considerable increase of speed might have passed unnoticed, because of the alternation of forward and backward walking.

But even if such an increase of speed should be found, it could hardly be as important as the swerve of the body, seeing that similar curves are made by an automobile where, as has been pointed out, variations in speed can hardly be considered as connected with the drift of the spiral turns, since the speed-controlling mechanism was not in the hands of the person steering. The spiraling mechanism seems, therefore, to have a rhythmical swerve component which is the direct cause of spirality in human paths; without it the paths might be a series of superimposed circles, but they would not usually be spiral.

One of the interesting things about the longer spiral paths is that they can be so well represented as projections of helical spirals on a plane surface. Take, for example, a regular helical spiral made of wire like a corkscrew, which makes spiral turns at an angle of 30° to the long axis. On looking along the long axis one sees a number of superimposed circles—a condition which occasionally is seen in parts of paths walked or swum. Then, if one raises the eye a few degrees (from 2 to 5°), a typical closely wound helical spiral is seen, as, for example, in *Ç5*. If one lifts the eye still further and looks at a larger angle (6 to 10°), a loosely wound spiral is seen, as, for example, in *Z56*. A view of the spiral model from still larger angles gives spirals of the *R87* type and some of the mice spirals. Looking at the model from a still larger angle gives a line of the cycloid type, but no paths are known in any animal of this kind. But if one looks at a still larger angle, that is up to 90°, the line is a sinusoid, which is the typical path of the ameba on a plane surface. It is, of course, to be understood that, viewing a helical spiral from any given angle is the same as drawing a projection of the helical spiral on a plane surface from that angle. Or, in other words, a projection is the shadow on a plane surface of the spiral model by light striking it at any given angle.

Now, the sinusoidal path of the ameba on a plane surface has been shown to represent actually a projection of a helical spiral path on a plane surface. There is every reason to believe that the path-directive mechanism in ameba functions for three-dimensional space, but that, because of physical limitations, the mechanism must confine its operations to two dimensions. But this does not destroy the operations of the mechanism; it continues to operate, 'doing the best it can' under the conditions, and the result is adequately described, so far as we can see now, as a projection of a helical spiral on a plane surface from an angle of 90°. The question therefore arises, Can we safely use this method of handling the walking and other paths of man? Specifically, the question is, Assuming that the path of man is the projection of a helical spiral on a plane surface, in what direction does the axis of the helical spiral lie?

In the ameba the direction of the helical spiral axis is parallel with the surface on which it moves, that is, it coincides with the long axis of the tube in which the ameba moves spirally. And the helical axis is also parallel with the long axis of the sinusoidal path. This is plainly indicated by observations, especially of amebas moving through glass tubes. Similar experiments in man, however, do not seem possible at present and the problem must be attacked indirectly.

In the first place, the axis of the helical spiral in man seems to be at right angles or nearly so to the chosen direction of walking, at least in those paths where a large number of spiral turns were made and where the handle was short and curving from the start. These paths are assumed to show the operation of the spiraling mechanism with least outside interference. If, in these cases, the walker immediately started on the helical spiral path in a way similar to that in which amebas and the ciliated organisms swim, then, in order that the axis of the helical spiral path may be at right angles or nearly so, the helical spiral must be closely wound, as close as 70°, with 90° as the limit. This is true only, however, if it is assumed that the spiral axis is not bent regularly at the start of each path, which would require postulating a new and additional factor. It is further certain that the helical spiral is not projected on the ground from directly overhead, for that, as has been pointed out, would give a sinusoid, but it must be projected at an angle varying from about 2° to about 12°, and, of course, in the same direction in which the axis of the spiral was propagated. In those paths in which the spiral axis was directed to the same side of the chosen path toward which the first complete turn was made, the handle of the helical spiral is supposed to lie on the ground, for the purpose of visualizing the projected path. Those spirals in which the spiral axis is propagated in a direction opposite to that in which the first complete turn was made, may be projected in the same way as the preceding by rotating the helical spiral on its axis about 180°, thus bringing the handle up into the air. Or one can visualize the two different kinds of paths by thinking of the former as projected against an up-hill plane and the latter as projected against a down-hill plane, from a horizontal plane in each case.

By varying the amount of rotation of the helical spiral on its long axis, projections within the angular range given above will give paths corresponding to all those observed. This degree of uniformity of fit of typical helical spiral projections to the observed paths when confined to the angular range stated seems to indicate that the helical spiral mechanism in man operates with closer turns than that of the ameba, resembling that of some of the ciliates (Bullington, '26) and paramecia and euglena when the temperature is lowered. In the latter case the spirals become more and more closely wound as the temperature is decreased (Schaeffer, '20 a).

### *The subjective element in spiral movement*

In these experiments on spiral movement in man the subject was of course conscious of some of his movements, and therefore the subjective element has to be taken into account in analyzing the data. The subject, moreover, in every case deliberately determined to walk a path of definite shape, either straightaway, circular, a square circumscribed about a circle, a figure-8, or a combination of two of these. It has been found impossible so far to devise an experiment which shuts out consciousness as a directive force completely, although counting by three's as rapidly as possible does this at times, but with no perceptible effect on the spirals except perhaps to decrease the size of the spiral turns. It is a striking fact, therefore, that, in spite of the necessary inclusion of this conscious directive element, no subject ever walked a path as directed by consciousness, and consciousness was never aware in any case of the nature of the path that was actually walked, swum, or driven. Consciousness seems wholly incapable of getting a direct report from the spiraling mechanism. The question therefore arises in how far consciousness influences the shape of the path. Is there evidence that it has any influence at all?

1. The paths are smoother in curvature when the attention is kept off the walking as much as possible by counting the steps and otherwise occupying the mind. Those persons who easily concentrate the mind on any subject before them and hold the attention on it, walk smoother spirals and fall into a spiral path more quickly than others. An increasing degree of smoothness is also noticed in the successive paths made by any particular subject; as the subject gains confidence the paths become smoother.

2. The straight handle to the paths where a straightaway is attempted seems to be due to the predominant activity of consciousness as contrasted with the activity of the spiraling mechanism. That is, the discrimination in the muscle sense or in the labyrinth is more acute at the start than later. Persons who concentrate on something other than the actual walking or swimming process, have shorter straight handles to their straightaway paths (excepting the heterotropic starts), and in a few cases none at all. That the conscious attempt to walk a definite path competes with the spiraling mechanism in directing the path and that the resultant path depends upon the degree of acuteness of discrimination of muscle sense involving the locomotor musculature, is best illustrated in those experiments where the attempt was made

to walk around circles of 31 meters' diameter. In these experiments where the reports from subjects were that they felt that they 'might be going off at a tangent,' the actual circles walked were considerably smaller than the marked-off circle of 31 meters. Where the opposite tendency was felt and reported, the path was circular at first, but of larger diameter, and almost at once became open with considerable stretches almost straight. It will be recalled that the maximum size circle that can be walked is about 40 meters in diameter, the mind being unable to discriminate between the muscle sense or labyrinth reports arising from walking along the periphery of a 40-meter circle and walking a straightaway. The degree of fatigue of these sense organs, whatever they are, is measured by the difference in the swerve required to walk a 40-meter circle and that required to walk any circle actually walked when attempting to walk a straightaway.

The experiments in which the narcotic drug was employed had very much smaller spiral turns than the normal paths, which was very probably brought about by decreased discrimination in the labyrinth or in the muscle sense or both.

3. Several experiments are recorded in which the subject, after having begun to walk in a spiral while attempting to walk a straightaway, stopped for ten seconds or more and then, on continuing his walk, proceeded along the curved path he was making at the time. In a few cases the blindfold was removed, a brief observation made, and then readjusted. When walking was resumed, the path was curved like the preceding section, but of somewhat larger arc. The effect of the spiraling mechanism held over in these cases, there being not sufficient time to establish that degree of sensory discrimination usually present at the beginning of an experiment.

4. The reports of subjects concerning the direction they walked, whether to the right or to the left, are unreliable, being as often wrong as right. A sample case is the following: "I felt I was driving (automobile) too far to the left, so turned the wheel and kept on turning it slightly to the

right to correct for it when I suddenly found I had turned the wheel as far as it would go and was actually trying to make it go still farther to the left.'' The subject was actually going in small circles to the left for some time. Two subjects, swimming, reported that they felt they were going in circles and seemed to be in the 'grip' of something that made it easier for them to continue going that way than going straight.

5. The spiraling mechanism can make any turns whatever in the path, even reversing the direction of turning from right to left or vice versa, without the subject being aware of it, so long as the turns are not so short as to permit of discrimination by the muscle sense or the end organ of the eighth nerve. The spiraling mechanism is therefore continually leading the subject around in two-dimensional space when consciousness 'thinks' the subject is going in only one direction. Or, in certain conditions, such as walking around marked-off circles the subject may actually be walking in one dimension when consciousness is 'aware' of going in two. Or the subject may be making a left turn when consciousness is reporting a right turn.

When walking straightaway the spiraling mechanism tends to make the circular turns as small as possible, which is about 7 meters in a few paths where a considerable number of spiral turns were made. In swimming it may be less, 5 meters, even as small as 2 meters. How small would the spiraling mechanism make the turns if consciousness permitted? Could the spiraling mechanism operate apart from the subject's consciousness of wanting to move on? And this raises the question for other spiraling organisms: Is a paramecium conscious of wanting to swim straightaway, or a sperm cell, or the male pronucleus as it makes its way through the cytoplasm of the egg toward the female pronucleus? No experiment has yet been devised which would help to answer this question for man.

6. Since the subject is not aware that he is walking any but a straight path while walking a straightaway, it follows,

of course, that the spiraling and reversal of direction of turning are the result of the operation of the spiraling mechanism, except in so far as it may be limited in its operation by the limits imposed by the muscle or equilibrating sense as reported in consciousness, which, so far as we know now, acts only in preventing too small a diameter of spiral turns. The gradually decreasing size of the spiral turns noticed in most of the straightaway walking paths is probably due to a gradual decrease in acuteness of discrimination of the sense organs of direction. This is also probably true for swimming paths. In the automobile paths the spiral turns are seen to be kept from getting smaller probably because of sensations received from the endolymph of the semicircular canals. It has been noted that smaller spiral turns would have been made by several of the subjects in driving automobiles if the steering gear had permitted it.

Now, as has been pointed out in the description of the experiments, the paths of the subjects are strikingly individual; they are as characteristic of the individual as any other activity. It follows, therefore, that the individual peculiarities of the paths are due either directly to the structure of the spiral mechanism or to another mechanism, the general nervous or brain structure, through which the spiral mechanism expresses itself.

7. An appreciable degree of physical fatigue after walking a considerable stretch resulted in smaller spiral turns in several experiments, which is what would be expected if decreased acuity in discrimination by the labyrinth or muscle sense permits of smaller turns. For it has been established that general physical fatigue reduces mental energy. But it is also possible that fatigue acts on the spiraling mechanism directly.

### *The individuality of the paths*

1. One of the most striking results of the study of spiral movement in man is the individuality of the paths. This characteristic was not at first apparent. In fact, it really did not become convincing until a considerable number of paths

of four of five individuals, under various forms of locomotion, were collected and analyzed. Moreover, short stretches of path are not sufficient. In the case of walking paths, for example, the paths should be at least 1500 steps long and preferably longer. It would, indeed, be desirable to have paths two or three times as long, but this need was not foreseen until very recently, and it is also something of a physical hardship for the average subject to walk that far blindfolded.

2. The individuality of the paths shown in this paper is best illustrated by the paths of M, W, Z, D, and F. A comparison of *M9, M10, M11, M12, M16, M19, M20, M44,* and *M45* reveal the following outstanding characteristics. Predominant right spiral of gradually decreasing diameter followed by an increase in the diameter of the spiral turns; in several paths which were long enough to show it, a series of smaller spiral turns with a following series of larger turns. That is, M's paths are a series of right spiral turns showing alternate decreases and increases in diameter without reversal of direction. (*M13* and *M14* are left turns ending against obstacles, and *M15* was walked when one ear was temporarily deaf.)

3. The outstanding characteristics of W's paths, *W29* to *W35,* are: A beginning series of small right or left spiral turns followed by a comparatively straight stretch. This is followed by another series of small spiral turns to the right. None of the paths are long enough to know in what direction the next series of spiral turns, if any, would turn. It is worthy of note that reversal occurs only if the first turns are left.

4. The paths of Z are characterized by a reversal of direction in every case between the series of spiral turns, as in *Z55, Z57,* and *Z58.* In *Z59* there is also a reversal. It will be noted that *Z55* is walking straightaway; *Z57,* steering an automobile; *Z58,* directing the steering of an automobile by calling the direction to the driver, and *Z59,* walking around a circle.

5. The paths of D are characterized by a long series of spiral turns, either to the right or to the left, of gradually

decreasing diameter, preceded by a long handle. Most of the paths show a near-balance between right and left turning.

6. The paths of F, of which only three are shown, have only a very few spiral turns and long sections of slightly regular or irregular curved paths. All the regular spiral turns are to the left.

7. Not only may the paths be arranged according to the individual who made them, but it is also possible to arrange the paths of all the individuals tested, in a series at one end of which are those paths with a very large number of spiral turns of gradually decreasing diameter, *C5, D61,* with no indication of reversal; and at the other end the very open paths with very few spiral turns and frequent changes of direction, as in *F71* and *I66*. In this series the paths of W come somewhere near the middle, with M close to W, but on the side toward the spiraling end, and Z also close to W, but on the other side, toward the open paths. This arrangement is possible only, however, if the paths of an individual are taken as a whole. For an individual occasionally walks a path which is markedly different from his other paths, for which a plausible explanation cannot always be found. An illustration of such exception is *D60,* a path which belongs much farther toward the open-path end of the series than all his other paths.

8. Now, it is a remarkable fact that there is a considerable degree of correlation between the type of temperament of a person and the character of the path that person makes. If one assumes that there are two fundamental types with respect to the handling of ideas, namely, those who concentrate on a particular thought or idea for a long time, the 'plodders,' and those who change comparatively frequently from one idea or thought to another, the 'temperamentals,' then the plodders are found to make paths consisting of long and regular series of spiral turns with gradually decreasing diameter and without reversal of turn. The temperamentals, on the other hand, make the open paths with few spiral turns, frequent reversals, and many nearly straight stretches. Much the

larger number of paths are, of course, made by intergrades between these two types. This correlation is very difficult to handle, owing to the lack of definite characterization of temperamental differences; but it is quite within the range of possibility that the further development of the relation between temperamental differences of the type specified and the character of the path, may give results of a quantitative nature which will help in understanding temperament. It is particularly important to remember that the character of the path is, so far as we know, entirely free from consciousness, that it is independent of the operation of the locomotor musculature, and that it is due to as deep-seated a mechanism as one may hope to find in an organism. If, therefore, a relation between this well-insulated mechanism and such a phase of activity as temperament exists, then here is at least one method of studying certain phases of temperament in a quantitative manner. It must be determined, first of all, whether the correlation is general only or whether there is a point-for-point relationship between the curve and the general intellectual or emotional process. For example, do the reversals correspond directly or only as a type form, to changes from one idea or thought attitude or emotional state to another? This correlation between temperament and character of path indicates that the different mind attitudes which characterize temperament have a subconscious or more probably an unconscious origin, which may be as deep-seated as the spiraling mechanism itself, or perhaps a phase of its activity.

### *The nature of the spiraling mechanism*

*Location.* The spiraling mechanism 'center' is not located in the locomotor organs, as the experiments with the automobile show clearly enough without bringing forward any other evidence. But the mechanism controls the motor system in a remarkably well-coordinated manner. So far as man is concerned, the center of the mechanism is, of course, somewhere in the brain. Is it in the coordinating center controlling the definitive locomotor system? It may be, as some experiments indicate, but evidently not according to others. In those experiments where the directions for driving the automobile are called out to the driver by the blindfolded person sitting beside him, the mechanism apparently must reside either in the cortex or in the sense organ (the semicircular canals?) which reported in the consciousness the deviation from the putative straight path toward the objective in which the automobile was to travel. Or did the locomotor center or the locomotor mechanism as a whole act as a sense organ in this case? What seems certain is that the spiraling mechanism begins to operate and then, some time after, the deviation in the path is reported in consciousness through some sense organ; but the spiraling mechanism is not necessarily in that sense organ nor directly connected with it.

In the lower animals, such as the unicellulars, the spiraling mechanism is located in a single cell, and in the higher animals it may function also in a single cell, the spermatozoa (Schaeffer, '20); and it operates in a fraction of a cell, as in part of a ciliate (Jennings). It also operates perfectly in the excised tips of tentacles from the brood pouch of the jellyfish Aurelia, which swim in the same direction as the sperm cells and the ciliated larvae from this animal. It is possible, therefore, that the effective spiraling mechanism may at any given time be located in a single cell in the human brain; for any mass of protoplasm which moves coordinately whether it consists of a part of a cell, a whole cell, a piece of an animal, or a whole animal, moves spirally. The conclusion seems inescapable that any cell or cells in the brain which act as a coordinating locomotor center will act on the motorium in such a way as to move the animal in a spiral path.

In this connection it may be permissible to digress for the length of a paragraph in order to point out the relation of some of this discussion to the concept of the unity of the organism. In most organisms a large part of the body is concerned in locomotion, and in coordinated movement this is always in a spiral path. Now, when one compares the very different ways in which different muscles are employed in the various swimming strokes, walking forward or backward or both alternating, and steering an automobile, and notices that the paths in all cases are spiral and nearly, if not quite, all of a typical individual pattern, one must be considering a unifying mechanism of very thoroughgoing efficiency. It is particularly interesting that parts of organisms (jellyfish) supplied with motor organs, but incapable of reproduction or other fundamental biological processes, should also move spirally, and, so far as the experiments have gone, turn in the same direction as the whole organism. The unity of the organism is thus exhibited from a new angle and involving details not touched by other activities.

*Relation of right-handedness and left-handedness to the spiraling mechanism.* It has already been pointed out that right-handedness is almost as frequently associated with left-footedness as with right-footedness, that there are varying degrees of right-handedness, and that right-handedness is an ambiguous term, being used by some to indicate a morphological difference (a longer or a heavier arm) and by others as being stronger, more 'clever.' As it happens, three of the subjects are classed as left-handed, physiologically, and these walked paths corresponding, in their spiral turns, to the majority walked by the physiologically right-handed. There is thus no definite relationship, if there is any whatever, between right-handedness or left-handedness and the dominant way in which the individual turns in walking blindfolded. The spiraling mechanism is more deep seated and is demonstrably present in more of the protoplasm of the body than is right-handedness or left-handedness.[10]

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[10] In a recent communication from Dr. Eugene Penard, celebrated student of the Protozoa, he cites some experiences of his that bear directly on this point. He is right-handed and left-footed. "When fishing for rhizopods in Lake Geneva I have been caught half a dozen times in dense fogs and generally, when deprived of a compass, found myself, after an hour's rowing, just at the place I had left; but curiously enough, the circle I was going in, was always to the right. On the other hand I was caught in fogs in the mountains at least three times and yet the result was the same, a right-hand circle."

It has been pointed out that there are frequent reversals from right turning to left turning and vice versa, in the paths, although the full significance of this phenomenon is not clear, owing to the lack of paths much longer than the longest I now have. But taking these records as they are, the total number of right turns is nearly the same as the left turns, although in the walking paths alone the number of right turns is nearly twice that of the left turns. A similar result was found by Dunkelberger in her observations on mice. Of the 2502 spiral turns which she recorded from 25 young mice, 1276 are to the right and 1236 to the left. Fourteen mice predominated in right turning and 11 in left turning. Of 12 noddy and sooty blindfolded terns let fly from Loggerhead Key Lighthouse, at Tortugas, Florida, 10 strongly predominated in making right turns and 2 in making left turns (Schaeffer, '27). But amebas (Rugipes bilzi), moving around glass rods or in tubes, move more frequently to the left than to the right in the proportion of about 2.1 to 1. Man, mice, terns, and amebas are the only animals so far known to reverse frequently from right to left and vice versa when spiraling. Oscillatorias and Spirillum reverse frequently the direction of progression along the spiral axis, that is, forward to backward and vice versa, but not the direction of spiral turning. The Atlantic palolo worm also reverses the direction of axial progression, but only once during its lifetime. As a ciliated larva it rotates to the left in a helical spiral, but the spiral turns get closer and closer together until, at six days of age, no forward progression is made. The larva then sinks to the bottom and begins to crawl. When it begins to swim as a worm, the tail takes the lead and the worm turns in the same direction, to the left throughout its whole life, but the direction of progression is changed. The zero point of change from forward to backward swimming occurred, in fact, at the end of the first six days of life as a ciliated larva.

In most organisms the direction of spiral turning is fixed, that is, a given species spirals either to the right or to the left in well-coordinated swimming, without reversing. Thus

Bullington ('26) examined 164 species of ciliates, of which 102 species always spiral to the left and 62 always to the right. And Prof. Frazier Cochrane (unpublished) studied 102 species of rotifers, of which 92 always turn to the right and 10 always to the left. Miss Old (unpublished) studied 35 flagellates, mostly of the Euglenoidina, and reports all of them always turning to the right. No other groups have been studied extensively. It seems, therefore, that those organisms having no highly differentiated guiding senses (eyes, equilibrating organs) which ordinarily rotate on their long axes, move in spirals with fixed direction of turn; while the others which ordinarily do not rotate on their long axis frequently reverse their direction of spiral turning. What is the significance of this?

Reversal of direction of spiral turning may occur without reversal of direction of progression in man and in the ameba; and reversal of direction of progression may occur without reversal of direction of spiral turning, as in the palolo. Those animals which possess a dorsoventral axis (morphological or physiological) and ordinarily move on a solid substratum, that is, in two dimensional space, or have good orienting senses, so far as the observations go, may frequently reverse the direction of spiral turn under experimental conditions. This holds true only for the stage of development of the animal of which these conditions hold. If there is a free-swimming stage (ciliated larval), the direction of spiral turn is fixed for that stage. How does the spiraling mechanism become changed so that spiraling remains, but an occasional reversal in direction occurs?

*Relation between spiral structure and spiral movement.* Spiral structures in animals and plants were known long before spiral movement was observed. The spirally twisted mollusc shells, vertebrate horns, and climbing vines were observed in earliest antiquity (compare spiral-shell ornaments of the Magdalenians), while spiral movement became known scientifically only since the advent of the microscope. But the number of spiral structures in organisms is very much larger than is perhaps commonly realized, and new spiral arrangements are constantly being discovered. A consideration of the connection between spiral structure and spiral movement narrows down to the microscopic forms. for no other groups have been studied from this point of view. In this field the most extensive observations were made by Bullington ('26), who examined especially the spiral structure of ciliates to see whether the direction of the spiral oral groove in their bodies influenced their direction of spiral turning in swimming. Of the 164 species of ciliates studied, 67 had distinct oral grooves which in every case turned to the right. Of the 67, 48 swim spirally to the left and 19 swim to the right. This shows that there is no apparent direct connection between spiral structure and spiral swimming in the ciliates. A similar lack of positive correlation is found in the flagellates. But in Spirillum and in the Oscillatorias, the direction of spiral twist of the filament is the same as the direction of spiral movement. And in the rotifers the same correspondence is observed in those few species of rotifers which have a spirally twisted body.

It will be noted, however, that, in the rotifers, Oscillatorias, and Spirillum, the entire body is twisted so as to become, as it were, a section of its spiral path, while in the ciliates and flagellates only a part of the body is spirally twisted, or only an organ, although this, like the oral groove of ciliates, is a large structure. It is easily observed also that the long slender soft-bodied ciliates, flatworms, etc., have their bodies twisted into a spiral segment that corresponds roughly with a segment of the swimming path. That is, the long axis of their bodies is tangent at a number of points at all times to the spiral swimming path. This is also true of stiff-bodied forms, but here the long body axis is tangent to the spiral path at only one point. This is an important consideration, since these organisms, like man and mice, can have neither their long axis (swimming) nor their axis of progression (man, walking) tangent to the assumed helical spiral path.

Now, it is a question whether the spiral bodies of the rotifers, Spirillum, and Oscillatorias are the result of spiral movement or whether spiral movement is caused by the spiral twist of the body. In the first place, there are many rotifers with straight bodies which swim exactly the same type of path as those with twisted bodies. The twist has not changed the general type of spiral swimming seen in the straight-bodied rotifers to any discoverable degree. But the twist of the body corresponds in direction and degree of curvature to the direction and curvature of the path. Moreover, the straight soft-bodied rotifers twist their bodies to follow the path after they start swimming, but of course straighten out again when through swimming.

In Spirillum the angle of spiral of the body corresponds with the angle of the spiral path and is always in the same direction. In Oscillatorias (Oscillatoria, Spirulina, Arthrospira) the angle of spiral movement is about 30° from the long axis in the straight filaments and a single turn in the coil of the coiled filaments corresponds, in length, to the length required for one rotation in a straight filament of similar diameter. This seems to indicate that the straight filament is under some kind of spiral torsional tension which is relieved as the thread becomes coiled. The coiling is done in such a way that the direction of turning in movement remains the same as in the straight threads. That is, if a coiled Arthrospira or Spirulina thread were uncoiled as one unrolls rope off a drum at right angles to the axis of the drum, and the thread retained the original character and power of movement, it would now move without rotating. But if the filament is uncoiled as one would slip off the rope over the end of the drum without letting the drum or the ends of the rope rotate, then the filament would move spirally at an angle of about 30°. If in the phylogenetic or ontogenetic process of coiling the straight filament with the usual spiral movement were rolled into a coil in the same way one wraps a piece of string or rope, which is in a left spiral, closely around a pencil, then, if the original character of movement were retained, the coil would now rotate in the opposite direction, that is, to the right. These conditions are easily illustrated in the manner indicated, or better still by cutting a short piece of rubber tubing of 10 mm. bore into a spiral band 3 or 4 mm. wide and stretching out the spiral band. All the conditions observed in the Oscillatorias indicate that the coiling conforms to the specifications of the spiral movement process in the straight filaments. It is worth noting, too, in this connection that coiling in the regular manner in which it is observed in Arthrospira and Spirulina and in some Oscillatoria could not occur without a definite bilateral spiral asymmetry in the filament, and that the line of this asymmetry is parallel with the direction of spiral movement in the straight filaments. It is conceivable, therefore, that there is a potential symmetry (bilateral asymmetry) in the finer structure of the straight Oscillatoria thread.

Bullington ('26) found a relatively high degree of correspondence in the direction of spiraling between the species of the various genera of ciliates, taking each genus by itself. Thus of the thirty-five genera containing two or more species, twenty-two genera had each all of its species spiral in the same direction, either right or left, and only thirteen genera in which one or more of the species spiraled in one direction and the other species in the other. The members of any one species, however, all spiral in the same direction. The spiral structures, however, in so far as they can be made out, turn to the right in all the ciliates except in the Peritricha, in which, with very few exceptions, the spiral structures turn to the left.

Now, if one divides all the ciliates into two groups: 1) those in which only right-spiral structures appear and, 2) those which have left-spiraling structures, then, using Bullington's data, there are 100 left-spiraling and 42 right-spiraling species in the first group—a ratio of 2.38 left-spiraling to 1 right-spiraling. And in the second group there are 20 right-spiraling and 2 left-spiraling—a ratio of 10 to 1. In each group there is, therefore, a definite negative correlation between the direction of turn of spiral structure and that of spiral movement.

A similar but more pronounced correlation exists among the flagellates. With only a very few exceptions, the spiral structures in the flagellates turn to the left, and it is possible that some of the exceptions are due to errors of observation. The spiral movements of some forty species of flagellates, belonging mostly to the Euglenoidina, have been studied carefully, and all these spiral always to the right.

These represent the only groups of organisms possessing definite unpaired spiral structures in which spiral movement has also been studied, and in these three groups, as has been seen, the spiraling of the group is more or less in the direction opposite to that in which the spiral structures turn.

This comparison between the direction of spiral winding of structures on an animal and the direction of its spiral movement also shows how thoroughgoing is the direction of the structural spiral in a group, being more definitely characteristic of the group than almost any other structure. This is also marked among the gastropod molluscs; and in the twisting of mammalian horns, where, with exceedingly few exceptions, the wild goats and antelopes have the right spirally twisted horn on the left side of the head (heteronomous) and the oxen and sheep have it on the right side of the head (hononomous) (Wherry, '02, British Medical Journ.). Then there is the large subject of leaf arrangement or phyllotaxy of the spermatophytes which engaged the attention of a large number of botanists in the past and more recently in the extended study by Church. In this field, where some of the most perfect examples of spirals exist, there is also a surprisingly high degree of conformity among the members of different groups. But spiral movement does not occur among the plants except among the flagellates, the zoospores and the motile gametes among the algae and aquatic fungi and the spermatozoids up to the cycads and the gingko among the gymnosperms. But almost no careful studies have been made of the spiral movements of these motile reproductive bodies from the point of view of the present paper.

Taking all these findings together, it is evident that the direction of turn of a spiral structure is very hard to change in the evolutionary process. It is much more readily suppressed than changed over to the other direction. In comparison, the direction of spiral movement is somewhat more easily changed in the evolution of a group, but spiraling is never suppressed nor appreciatively reduced in effectiveness: it is either left or right. That there is a negative correlation between the direction of spiral and that of spiral movement indicates that the two phenomena are connected in some way, but how remains to be discovered.

*Sizes of spiral turns in various organisms.* A comparison of the sizes of the spirals with the sizes of the animals shows that there is a direct general proportion between size of spiral and size of animal as is shown in the following table, all measurements being reduced to microns for the sake of ready comparison.

TABLE 4

*Showing ratio of body length to length of spiral turn in various groups of animals*

| NUMBER OF SUBJECTS OR SPECIES | KIND OF ANIMAL | LENGTH OF SPIRAL TURN IN BODY LENGTHS OF ANIMAL | LENGTH OF ANIMAL IN MICRONS |
|---|---|---|---|
| 37 subjects | Man, walking (*C5*) | 11 | 1,771,580 |
| | Man, swimming (*D55*) | 5 to 8.5; smallest is 2 | 1,771,580 |
| | Man, automobile | 20 | 3,362,000 |
| 12 subjects | Tern, flying | 41; smallest, 32 | 228,591 |
| 2 subjects | Toadfish | 2.5 | 176,394 |
| 25 subjects | Mice | 8 (average); smallest is 4.5 | 44,448 |
| 31 species | Ciliates | 4.41 | 172 (average) |
| 102 species | Rotifers | 4.85 | 370 (Philodina) |
| | Paramecium aurelia | 4.5 | 300 |
| | Coleps hirtus | 4.0 | 66 |
| | Euglena viridis | 5.0 | 50 |
| | Chilomonas paramecium | 4.0 | 27 |
| | Spirochaeta stenospira, 1 spiral | 5.0 to 6.5 | 1.2 |
| | Ameba (Rugipes bilzi) plane surface | 5.0 to 8 | 70 |

This table shows a representative list of animals on which quantitative observations have been made with respect to the number of body lengths per spiral turn. It is at once evident that the range in size of the animals is very much larger than the range in the number of body lengths per spiral turn. This ratio is, in fact, practically a constant for the lower forms, namely, about 4.5, when averaged for small taxonomic groups. It is also evident that the large number of body lengths per spiral for the terns is due to the speed with which they have to fly to keep up in the air. Speed likewise enlarged the spirals in which the automobile was used. The swimming and walking spirals selected represent the largest number of uniform spirals in any one experiment of either class. A few much smaller spirals are recorded in *L68*. In measuring the diameter of the walking and swimming spirals, it was arbitrarily assumed that the length of the spiral turn equaled the circumference of the spiral turn. It is also to be noted that in this table the length of the animal is perhaps as fair a criterion as any to use for the purpose of comparison, but it evidently would not be satisfactory for very slender animals like worms or some sperm cells, for example. It is doubtful also whether the mass of the animal could be taken as a basis because of the great difference in effectiveness, size, and distribution of the motor organs. The fact that the long slender ciliates swim a smaller number of body lengths per spiral turn and at least some of the spherical a much larger number indicates that the product of length and one-half the sum of the long and short diameters would probably give a truer measure than length merely. But it is also true that slow swimming shortens the spiral turn, and the symmetrical distribution of the cilia undoubtedly influences speed. It is, however, impossible at this time to introduce a correction for all these factors, especially the last two, for the observations are not nearly extensive enough. Length has therefore been used as the best single measure, and it gives a high degree of uniformity of body lengths per spiral turn when applied to the observations on ciliates, flagellates, and rotifers statistically.

When one considers a group of organisms like the Oscillatorias, the Spirochaets, Spirillum, and the motile filamentous sulphur bacteria, then the length of the organism is no longer of use as a measure of the amount of spiral rotation, for the length of most of these organisms is indeterminate. The diameters are practically uniform within the species and seem to vary no more than the length of ciliates varies. So this can be taken as a measure, but the results from using this measure cannot be compared directly with the other data from the ciliates, flagellates, and rotifers, for the average length of these animals is somewhat greater than their width, by perhaps 50 per cent or more. The great diversity of body form prevents arriving at a reliable ratio of length to width or to diameter. But it is possible in this group to measure with a fair degree of accuracy the angle at which the filament rotates on its long axis, and the angle at which the coiled filaments coil themselves up into spirals. Unfortunately, however, this exceedingly interesting group has been studied only very little from this point of view, but certain other observations have been recorded which are of great interest.

Spirochaeta stenospira (Zuelzer, '11), which is included in the table above, is one of the most narrowly wound spirochaets known. The filament has a diameter of 0.25 μ and the length of a spiral turn is 1.2 μ, along the straight axis with which the inside of the filament is in contact along its whole length. That is, the filament is wound around a line, as it were, leaving no space. The inside of the filament is therefore wound at a zero angle of curvature, while the outermost edge makes an angle of about 62° with the axis, and the geometrical axis of the filament makes an angle of about 32° with the axis of the coil. The Oscillatorias which have been carefully observed in movement also rotate at an angle of about 30° or slightly greater, and all the careful observations agree in stating that the rotation is to the left.

The observations on the small Spirochaet are of very great interest, because it is important to know in how small a mass of living matter the spiral mechanism can operate, and so far as known this organism furnishes the best answer available. A complete spiral turn is found in a helical spiral space 0.25 μ in diameter and 1.2 μ long. The finer structure in the protoplasm must therefore turn 1° in 3.47 millimicrons. This is within the range of molecular dimensions, but the rotation is considerably higher than the highest degree of rotation observed in optically active substances in the polarimeter (Vorländer and Huth, '11) where in liquid crystals of one of the amylesters a rotation of 11° per micron thickness was observed, or 1° in 99 millimicrons.

A direct comparison between these two cases of rotation cannot yet be made, in so far as the ultimate elements (molecules?) are concerned. In optically active solutions and in solid and liquid crystals the diameter of the solution is without effect if the depth, concentration, and temperature remain uniform. In organisms, however, a change in diameter changes the amount of rotation per unit (absolute) length of organism.

Now, in this group of organisms the angle of rotation in movement in the straight filaments is, as stated, about 30° or slightly greater, never less. The angle of spiral coiling, when present, is in almost every case greater than 30°, most frequently from 60° to the closest spiraling possible, which is about 82° in the case of a Spirochaet; but it is somewhat larger in the Spirulina and Arthrospira genera where the coils have open spaces around the axis of the coil. Among the ciliates and rotifers, only the very rapid swimmers, with a diameter of spiral below the average, make a path of less than 30°; the great majority swim at angles of 30° or greater. When the temperature is lowered or conditions arise that are adverse, the angle of the spiral path becomes greater. Similar conditions are observed in birds and men. A sick tern, blindfolded and set free from the top of the Loggerhead Key Lighthouse, flew in perfect spirals, as it slowly descended to the ground, that were less than 3 meters in diameter (about 32 body lengths) as it reached the ground. Fear or preoccupation of mind in man, when blindfolded, causes slower walking and markedly smaller spirals. In other words, the spiraling mechanism constantly tends to produce spirals of larger angles of curvature and, in going down the scale of organisms, this tendency is present in the smallest spirals known, which are in the Spirochaets. The fact that the majority of organisms move in spirals at an angle in the neighborhood of 30°, when in coordinated locomotion, may indicate that the elements of the finer structure of the protoplasm concerned in movement are spirally arranged to the extent of about 30° or more, with respect to the diameter of the spiral path of the organism, or vibrate into this position more frequently than into any other. That the spiral path should constantly tend toward a larger angle when forward movement is interfered with to some extent by any one of various causes, indicates that the axial component of progression is dissociable experimentally from the spiral or rotational, and that the molecular or atomic structure of one or more of the substances involved in movement is spiral in form at a larger angle than 30° with respect to the path of the organism.

It is generally recognized that molecules may be built upon a spiral plan. For example, the carbon atoms, which are usually represented as tetrahedrons, 'to represent the four valences either on the apices or on the faces, probably arrange themselves more easily spirally to produce a compact-chain form than in any other order. Since a carbon chain (and an occasional atom that is asymmetrical) is one of the characteristic components of the molecules of amino-acids, proteins, and other carbon compounds in protoplasm, there is no lack of spiral arrangement in the finer structure of protoplasm to satisfy preliminary requirements of a spiral locomotor mechanism.

Now, the fact that in the simplest organisms the direction of structural spiral turn and of spiral movement is the same (i.e., both right or both left) may indicate that the molecules have no marked translatory or rotational movement, but chiefly vibratory, with the molecules partially oriented, however, as in the mesomorphic state (liquid crystals) seen in a number of carbon compounds. The spiral movement of the organism would then be the statistical result of the spirally vibratory molecular movements, and therefore in reality a byproduct of the fundamental metabolic activities.

In higher organisms, where there are motor organs and 'centers' for spiral and other movements, the problem is more complicated and probably similar to the central ontogenetic process. This atomistic view of spiral movement has the merit of being experimentally testable at almost every point. One or two of these points will be taken up a little further in a forthcoming paper on spiral movement in amebas.

The fact that the number of body lengths per spiral turn is so uniform among so wide a variety of organisms is undoubtedly of profound significance and must mean that when the motor system is functioning at what may be called its optimum speed, the spiral structure of the coordinating center of movement compels a spiral path of about 30° or a little larger. This is the average minimum effect at average speed. But if, through some cause the rate of movement is slowed down, the angle of the path becomes larger with a maximum of 90°, which means cessation of forward movement along the spiral axis and perhaps in some cases reversal of progression along the axis.

The time element seems to affect the angle of spirality in movement only markedly when it applies to speed, not when applied to the amount of time required to make a spiral turn. For amebas move spirally around glass rods and in tubes at only a slightly larger angle when the diameter of one tube is eight times as large as that of another, requiring a correspondingly longer time to make one spiral turn (Schaeffer, '26). This shows that the spirals are not laid down in miniature as it were, in the coordinating center, as movement proceeds, but must be regarded as based on a highly elastic structure which the dynamic phase of the motor mechanism is obliged to follow; unless one takes the view that the effect of the asymmetry of, say, the carbon atom, is overcome to some definite extent as a larger compound or structure is built up (or broken down) concordantly as movement proceeds at optimum speed; and that when movement slows down, the structure in question tends more and more to assume a shape corresponding to the inherent asymmetry of its smaller components. The former view seems more attractive, for the spiral structure and spiral movement agree in the lowest organisms in both the angle of curvature and in the direction of turning.

In casting about for an asymmetrical or spirally oriented substance in the protoplasm of organisms, one naturally turns to the results of investigations with the polarimeter on organic stuffs, especially amino-acids. Most of these are levo-rotatory, only a few being dextro-rotatory. But in this case levo-rotatory does not mean that the rays of light are turned to the left as they go through the solution examined, that is, that the path is a left helical spiral. The light rays are actually twisted to the right, but on the dial of the instrument the analyzer has to be turned to the left to match the color fields. If one refers, therefore, to the actual progress of the rays of light through the solution, the levo and dextro qualities of the solution as printed in the tables are just the opposite of what the paths of the light rays are. Now, an examination of the rotatory power of amino-acid solutions in polarized light shows that the rotation is very much less than is required for the degree of spirality observed in so small a space as one twist of a Spirochaet. In the Spirochaet, as has been pointed out, one spiral turn of 360° requires only 1.2 μ, while in the amino-acids the highest amount of rotation is only a very small fraction of this quantity (in all of them less than 360° in 10 cm. of solution). Thus the observations from polarimetry of solutions throws little light on the spiraling mechanism at present. But there nevertheless remains the possibility that further investigation will show that these two sets of phenomena depend ultimately on the same mechanism.

But, assuming that there is a spiral structure as a basis for spiral movement, the question arises as to how it is translated into the movements of the motor organs so as to make the organism's path a spiral. We have, of course, no answer to this question. It is similar to the question as to how a gene develops ontogenetically. The question of the means of translation of the spiral mode of movement from the 'center' to the motor organs is a part of a large general problem, including the ontogenetic process. A study of spiral movement seems particularly favorable for looking deeper into this general problem, because of the universality of the reaction and the quantitative definiteness with which it expresses itself in many groups, and because of the probable absence of any important change in the phylogeny of the fundamental mechanism.

*Bilateral asymmetry in movement.* There is one more relationship between movement and structure which this investigation has brought out that calls for comment. In man and most higher animals we speak of bilateral symmetry and, so far as the locomotor organs are concerned, the two sides are substantially alike structurally, although, so far as locomotion is concerned, this is not the case at all. At any given moment in locomotion, man, birds, mice, etc., are definitely right-turning or left-turning, when the orienting senses are not functioning. This asymmetry is capable of reversal in most of the birds, mice, and men studied. In rotifers, ciliates, flagellates, etc., as well as the ciliated larvae of many marine invertebrates, locomotion is similarly bilaterally asymmetrical in that the outer side of the spiral is a structurally definable side; that is, a particular side of the animal is always on the outer side of the spiral. This, of course, corresponds to the movements of a man, bird, or mouse when the right side, for example, of one of these animals is on the outer side when walking a spiral. But this bilateral asymmetry extends apparently down to all the motile organisms, including the Oscillatorias, Spirochaets, Spirillum, and possibly also the motile bacteria. In the coiled Oscillatorias, Arthrospira and Spirulina, the outer side of the coil is in all cases motile, while particles on the inner side seem to remain motionless. It has already been shown that one coil of these algae is the equivalent of one spiral turn as measured in locomotion in the straight species. Additional observations of Zuelzer ('11) tend also to show that the whole outer surface of at least one species of Oscillatoria is not motile, but only a spiral band running around it. Or there is a spiral band which moves more rapidly than the rest of the surface. This group of algae, then, which appears symmetrical from inspection of its structure, is probably functionally asymmetrical in locomotion. The Spirochaets have, of course, long been known to be bilaterally asymmetrical, having a more deeply staining rod along the inside of the spirally twisted body. The motile bacteria have, unfortunately, not been investigated from this point of view, but a few observations made on curved free-living bacilli, whose bodies are sections of a helical spiral, rotate in the same direction as their bodies are built and thus conform to the conditions found in the Spirochaets. This widespread correspondence in bilateral asymmetry of spiral locomotion extending to all the groups studied is of very great interest and must be connected ultimately with the finer structure of the spirokinetic mechanism.

## SUMMARY

1. When blindfolded persons walk or swim in what they intend to be a straight path, 300 steps or more in length, they actually walk in a more or less regular clock-spring spiral. The same is true when driving an automobile blindfolded or when a blindfolded person in an automobile calls the direction to the driver. The diameters of the spiral turns in walking and swimming varies from about 36 meters to about 6 meters, in automobile driving from about 100 meters to about 12 meters. The drift of the spiral turns is in nearly every case in the same direction in which the first turn was made.

2. A spiral path is also made when walking backward and when walking forward and backward alternately. In both

these cases the spirals are smaller than when walking forward only, but the drift of the spirals is frequently in the direction opposite to that in which the first spiral turn was made.

3. The spiral turns are not due to asymmetries in the legs or other parts of the locomotor organs or of the body generally, such as right-handedness.

4. One and the same person may walk or swim or drive both right-spiral turns and left-spiral turns in different experiments or sometimes in the same experiment. No apparent difference is observed in the direction of turning that is referable to differences in age, sex, or right-handedness or left-handedness.

5. There is marked individuality in the paths which different individuals make in the size of the spiral turns and in the number of reversals from right to left turning and in other characteristics. These differences seem related to fundamental types of behavior, and there is evidence tending to show that the open, more or less irregular spiral paths with frequent reversals in direction of turn are made by the more or less 'temperamental' persons, while the 'plodders,' on the other hand, make long series of spiral turns with few or no reversals. More subjects have to be investigated, however, before this can be fully established.

6. The mechanism which causes the spirality of the path in man must be located in the central nervous system, probably in the 'center' of one or more cells that coordinates locomotion at any given moment.

7. Spiraling is not a conscious phenomenon. Consciousness gets no direct report from the spiraling mechanism. A blindfolded person is 'conscious' of swimming or driving a straight path when he is actually going in spirals. The spirals are smoother, however, when the attention is not intensely directed on the manner of making each step, but is occupied in counting the steps or in thinking about something else than walking a 'perfectly straight path.'

8. A manic depressive and an imbecile, when blindfolded, walked essentially the same kind of straightaway path as normals.

9. Persons who lose their way in forests, snow-storms, fogs, etc., go 'round in circles,' because in these persons the orienting senses are not functioning, and then the spiraling mechanism guides the path. Rabbits, foxes, antelopes, and other game, when hard pressed in the chase, lose the power of orienting themselves through fear, and then the spiraling mechanism also leads them around 'in circles.'

10. The spiral path of man is interpreted to be the graphic expression in two dimensions of space of a helical spiral 'pattern.' Since it is not possible to ascertain definitely whether the spiral axis of the actual path points up or down, it is not possible to say definitely whether a right turn in the actual path is a right turn or a left turn in the helical spiral pattern.

11. The spiral path of man seems to be produced by the same mechanism which produces similar paths in blindfolded birds and mice and in rotifers, ciliates, flagellates, amebas, Oscillatoria, Spirochaets, ciliated larvae of many marine invertebrates, spermatozoa, zoospores, spermatozoids, etc., and is, in fact, just a special case of the general phenomenon that all motile organisms move in a helical spiral or a projection thereof on a plane surface, when not guided by orienting organs.

12. When all of the 697 spiral turns of thirty-seven persons, who set out to walk or swim straight paths blindfolded, are grouped into left and right turns, the right predominate over the left in the ratio of 1.48 to 1. If the direction of each experiment is taken, the ratio is 1.38 to 1. In the ameba (Rugipes bilzi) the left turns predominate over the rights in the ratio of 2.1 to 1. In the ciliates, where there seems to be a condition approaching chance distribution of left and right species in small groups, there are 102 lefts to 64 rights (Bullington)—a ratio of 1.64 to 1. In the only other groups of which data are available, the rotifers and the flagellates, the

right to left ratio is 9 to 1 in the rotifers and over 100 to 1 among the flagellates (Euglenoidina and Dinoflagellata).

13. Spiral structure and spiral movement in the lowest organisms, Oscillatorias, Spirochaets, and Spirillum, agree in the direction of spiral turn; that is, if the structure is left spiral the movement is left spiral, a spiral turn of the structure equaling one spiral rotation in movement. But in the ciliates and flagellates (Euglenoidina and Dinoflagellata) the correlation is negative, the right structure (oral groove) in ciliates being associated with left turning 2.38 times as often as with right turning, and in the flagellates left structures and right rotation seem to be almost universal.

14. The number of body lengths per spiral turn is practically constant among the lower groups (ciliates, flagellates, rotifers, amebas), being about 4.5 or perhaps somewhat less. In man, walking, the smallest regular spiral turns are about 11 body lengths; in swimming, 2 to 8.5 body lengths; in driving automobiles, 20 body lengths (of man). In terns, flying, the smallest spiral turns are about 41 body lengths.

15. The angle at which helical spiral turns are made by organisms swimming in three dimensions is in the neighborhood of 30°, or a little larger for the great majority of organisms. Very rapid swimmers (ciliates) make a somewhat smaller angle, and very slow swimmers make a larger angle.

16. The element of time is of comparative unimportance when it does not affect rate of movement; a spiral turn of the usual size will be made when interrupted by short rest periods (man) or at only a slightly larger angle if the diameter is increased over eight times (ameba, Rugipes bilzi).

17. The smallest space in which the spiraling mechanism has been found to function is one turn of a helical spiral of a Spirochaet 0.25 μ in diameter and 1.2 μ long, in which the substance along the spiral axis turns 1° in 3.47 millimicrons. Since there is good reason to suppose that the mechanism can operate in a smaller space than this (flagella of bacteria), it is clear that the structural basis for the spiraling mechanism may be and probably must be of molecular dimensions,

possibly a spiral chain form which depends for its form on the asymmetry of the carbon atom.

18. This investigation has also brought out the interesting relation that there is present in perhaps all motile organisms a locomotor bilateral asymmetry, which means that at any given moment an organism is either strongly right-turning or strongly left-turning, with a structurally definable side as a reference point. This asymmetry is reversible in those organisms confined to move in two dimensions of space, but fixed for apparently all other organisms. In Oscillatoria there seems to be only a spiral band which is motile, not the whole surface as is commonly thought, or else the spiral band moves faster than the rest of the surface. The dynamical basis for this phenomenon must depend apparently upon the structure of the spiraling mechanism.

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# A STUDY OF THE TONSILLAR DEVELOPMENTS IN THE LINGUAL REGION OF ANURANS

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TWENTY-SIX FIGURES

AUTHOR'S ABSTRACT

In this study the problem of the tonsil is considered in anurans. The common toad (family Bufonidae) is used as the type, and representative species of the other families are compared.

In each species representative stages beginning before transformation were selected, and the lingual region of each was sectioned. Some thyroid-fed toad tadpoles which had prematurely transformed were examined. The investigations led to the following conclusions.

1. Accumulations of lymphocytes occur in all the families except Hylidae.
2. A pair of tonsils located on either side of the tongue appear before transformation in Bufo, and persist, increasing in size through old age.
3. This pair has its developmental origin at or near the cephalic end of pouch II. In no other species examined do the tonsils appear as early; in almost all forms the accumulations are inconstant in occurrence, as are also some in Bufo.
4. The cells of the 'tonsils' are lymphocytes of varying sizes. They arise from the mesenchyme; later their accumulations become sites of lymphopoiesis.
5. This type of lympho-epithelial mass is simple in structure and has a greatly thickened epithelium, due to extensive infiltration by lymphocytes. In the connective tissue the vascular supply is abundant.
6. The differentiation of lymphocytes may be due to a factor of strain, arising through adjustments made during metamorphosis.
7. Thyroid-fed toad tadpoles transform, apparently without developing tonsils.

## CONTENTS

Introduction .......... 400
Materials and methods .......... 405
Tonsillar development in the lingual region of Bufo (type) .......... 407
Morphology and morphogenesis .......... 409
Histology and histogenesis .......... 412
Developmental origin .......... 416
Effect of thyroid feeding .......... 417
Tonsils in representatives of other anuran families .......... 418
Discussion .......... 425
General considerations .......... 430
Conclusions .......... 431
Literature cited .......... 433

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## INTRODUCTION

*Problem of the tonsil.*[1] The solution of the problem of the tonsil has been attempted by many workers. Tonsillar structures in mammals, and more especially in man, have attracted the greatest number of investigators, as would be expected. Comparatively little has been done with them in the vertebrates lower than mammals.

*Definitions and limits of the term.* There exists considerable uncertainty and indefiniteness as to the exact meaning of the term 'tonsil,' and it would therefore be advisable to define and limit it for the purposes of this paper. The common use of the term makes 'tonsil' apply only to the palatine tonsils of man. From an histological standpoint, however, one may consider tonsils as structures associated with epithelium and composed of lymphatic tissue. They consist essentially of connective tissue, epithelium, and lymphocytes. The former is the underlying tissue, more or less filled with leukocytes, mainly of the lymphocyte type. The epithelium is a limiting surface tissue, which may be, and usually is, invaded with lymphocytes. Ordinarily, the connective tissue is very vascular, but not necessarily so. Such a definition of 'tonsil' permits one to speak of intestinal tonsils, pharyngeal tonsils, and even those of the respiratory tract. The discussion of these structures in Amphibia, however, will be restricted to those of the pharyngeal and oral regions, and this particular study will be limited to 'tonsils' of the lingual region.

In the problem of these interesting structures, there are five salient aspects or phases that meet every worker: 1) the consideration of their occurrence, not at just one period of the animal's life, but during its entire existence; 2) the morphology and morphogenesis; 3) the histology and histogenesis—an important phase of the problem; 4) their origin; 5) the relations of these lymphatic structures to the problem of the lymphocyte.

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[1] The study presented in this paper was suggested to me by Dr. B. F. Kingsbury. The latter has kindly loaned me many series of slides of toads' heads. I wish to express my appreciation and gratitude for his help and encouragement while working upon this problem. Members of the Zoölogy Department at Cornell University have given me preserved materials of the forms which are difficult to obtain. Dr. A. H. Wright has been most generous with his anuran material in all stages of development. The Middle-western Scaphiopus specimens, and individuals of Ascaphus were provided by Mr. W. Senning, of Cornell, and Mrs. Gaige, of the University of Michigan, respectively.

*Occurrence and character in other vertebrates.* A general statement of the occurrence and character of the tonsils of the pharyngeal region in all vertebrate groups, as far as is known, will give a background for the more detailed description of them in the Anura.

*Mammals.* In general, the types of tonsils which will be enumerated are the ones which occur in the pharyngeal and oral regions of mammals—the palatine, the lingual, the pharyngeal, and the laryngeal tonsils with a fourth location, often, in the lower part of the eustachian tubes, called 'tubal' tonsils. To characterize them briefly, it would be sufficient to say that they reach quite a high degree of development and complexity in the group, and definite germinal centers are recognizable in the particular connective tissue. There is usually present a distinct membrane or capsule of connective tissue of a fibrous character that limits them on their inner surface. No one of these tonsils is always present.

*Birds.* As might be expected, the distribution of these lymphatic bodies is somewhat different among birds. There are five main locations that may be cited (Jolly, '23): 1) Lymphatic nodules occur in the connective tissue of glands at the level of the jaw articulation and the laryngeal orifice. 2) More diffuse accumulations occur in the region of the palatopharyngeal fossae. 3) The most prominent mass occurs in the region of the tubal infundibulum forming a lymphoid ring around the latter (Oppel, '00, and Jolly, '23). 4) There are a few lymphatic masses in the lingual region. 5) Lastly, some are to be found associated with the mucous glands in the pharynx. In birds, the so-called 'tubal' tonsils (3) are the most definite and best developed and may be as complex in their structure as the palatine tonsils of man.

*Reptiles.* Among the reptiles quite a variation in the position of tonsillar patches apparently exists. In the crocodiles, (Killian, '88) there are 'tubal' tonsils. In Lacerta (Oppel, '00) and related forms, pharyngeal and pterygoideal lymphatic accumulations occur. Hatteria (Oppel, '00) has only palatine tonsils. Thus it is evident that, although such lymphatic structures exist in reptiles, they are not numerous. They seem poorly distributed in the oral and pharyngeal regions and are indefinite and simple in structure, sometimes being recognized only with difficulty.

*Amphibians.* This is the lowest vertebrate group in which tonsils occur, as far as the author can ascertain. In it are found all degrees of subepithelial lymphocyte accumulation, from none at all, or a very diffuse condition, to structures which are quite definite and localized. An example of the first extreme is found in the case of some frogs in which the accumulations are very inconstant in presence and location. The toad (Bufo) and Salamandra atra are examples of the other extreme.

*Fishes.* Among the fishes, the lymphocyte accumulations are too diffuse and inconstant to be considered 'tonsils,' even according to the definition that has been given here.

*Review of literature on amphibian tonsils.* Considering first the urodeles, Holl ('85) was the first worker contributing any definite statement concerning the occurrence of tonsillar material. While he was making a general study of the histological structure of Salamandra maculata, he incidentally discovered tonsillar tissue, and noted that there was a pair of fairly well-developed structures, definitely and symmetrically located in the dorsal wall of the pharynx. The connective tissue in the region of these organs was highly vascularized. Other more diffuse and less constantly occurring and localized structures of this nature which he found in the mucous membrane he did not consider as tonsils. In 1899, Oppel, in Proteus anguineus, noted quite constant but diffuse tonsillar patches back of the jaw articulations. Compared with the sort of tonsil found in mammals, the one pictured by Oppel seems very diffuse, but it has the necessary characteristics for such a structure. The lymphocytes have invaded the epithelium which is several-layered, much thickened, and more or less reticular in appearance below the surface. He noted lymphocytes, or, as he calls them, 'wandering cells' in other locations, but they were not numerous or constant in occurrence.

In 1912, Kingsbury published a paper upon amphibian tonsils in which he made a particular study of Salamandra atra, among the urodeles. He found twelve localizations in the two regions we are considering. Of these, the following three patches were definite and fairly constant: the preglottideal (in front of the glottis), the lateral (near the articulation of the jaws), and the sublingual (located as the name implies).

He also made a comparative but less detailed study upon Desmognathus fusca, Diemyctylus viridescens, Plethodon cinereus, Amblystoma punctatum, and Gyrinophilus porphyriticus. In general, he concluded that almost all salamanders have tonsils in about the same region in each case. Desmognathus fusca and Gyrinophilus porphyriticus seemed to be exceptions.

The latest worker, Jolly ('19) gives a general statement of the sort of tonsils found in urodeles, but does not specify in which they occur, nor give details of his work. He says that the crypts, characteristic of these structures in man, anura, etc., give way to papilliform protuberances which are infiltrated with lymphocytes in the case of salamanders.

As to the character of the tonsils themselves, in the tailed amphibian group, there is found a fairly simple structure as compared with those in mammals. The connective tissue is a loose reticulum, with a considerable sprinkling of lymphocytes in its meshes. There is a well-developed vascular supply. The epithelium is several layered, and usually the surface is composed of the modified columnar or cuboidal type of cell, while the deep ones are irregular and somewhat reticular. The inner limit is usually recognizable. A denser accumulation of lymphocytes is found in the sublingual tonsils of

Salamandra atra. Veins and venules are always associated beneath or at the site of the patch, and usually these vessels are fairly well filled with lymphocytes. Kingsbury ('12) says that he has noted mitotic figures in these lymphatic patches, but they are not numerous enough to assume a rapid proliferation.

Among the anura there are very few descriptions of specific nature. Here, again, Holl is the first worker of importance. In 1887, he worked upon Rana temporaria, and discovered some accumulations of lymphocytes, or what he called 'Wanderzellen,' on the tongue and in the roof of the mouth. Drzewina, in 1905, mentions leukocyte infiltrations in the oral regions of the frog, but describes them as being quite diffuse. Kingsbury ('12) studied the tonsils of anurans, using the toad (Bufo americanus) as his type or example. He found in this form that 'sublingual' tonsils are of constant occurrence. He examined many individual heads at the time of completion of transformation, before it, and slightly after that stage. Jolly is again the latest worker. In 1919, he notes the occurrence of 'lympho-epithelial' masses in Rana temporaria, in the lateral recesses of the nasal fossae. He mentions that, in the toad, in the whole oral pharyngeal cavity, lymphatic tissue occurs in relation to epithelial crypts. Further, he describes 'palatine tonsils' in the jugomaxillary arc, in R. temporaria and in R. esculenta. These are the most constant and best developed tonsils of the forms mentioned.

Further than this no work has been done upon amphibian tonsils, so far as the writer is aware. This group of lower vertebrates is particularly interesting and desirable for study along this line, because of the comparative simplicity of the members, and has been chosen in the belief that the interrelations of structures here would not be so complex as in higher forms.

*Scope of this study.* The purpose of the following study has been twofold: 1) to ascertain the occurrence and general character of the lingual and sublingual tonsils, among the representatives of all the families of the anurans; 2) to undertake a more detailed study of this same group of tonsils in Bufo. In the latter case, attention has been given to the phases of the problem of the tonsil already mentioned: e.g., 1) occurrence; 2) character at representative stages; 3) morphology and morphogenesis; 4) histology and histogenesis, and, 5) their origin. The problem here, as is the case in all studies of the tonsil, has been that of the lymphocyte itself—why it occurs at these sites and under what conditions.

## MATERIALS AND METHODS

In making this study, representative genera of each family of the anura were chosen, and material of an available species of each of these was used. In all cases except in the genera of Bufo and Ascaphus, individuals of each species at the following four stages were examined: before transformation, at transformation, shortly after this stage, and the adult. The species of Ascaphus are comparatively rare in the United States, and the writer had individuals of only the stages before transformation, after transformation, and adult. In the species which are common locally, a large number of specimens were examined. In all other cases comparatively few individuals were available for this purpose. The writer's experience has been, however, that the presence or absence of tonsillar material in adults is constant, so that large numbers of individuals are not necessary to check the mere occurrence of the tonsil. The genus Bufo was used as the type for the whole group of anura, and many specimens at all stages from the early larva to a 97-mm. adult were examined. The largest number of individuals were examined in the period including stages just before transformation, at transformation (e.g., when the tail stub has disappeared and both the fore limbs have erupted), and immediately after this period. Most of the material of forms other than Bufo was from preserved specimens, and had been fixed either in 10 per cent formol or in alcohol. When this was the case the best results seemed to be obtained by further fixing the desired region (e.g., floor of the mouth) in Bouin's fluid for twenty-four hours before embedding and sectioning. It was found convenient to stain the region removed, in toto, with Mayer's acid carmine for five to seven days. Alcoholic eosin was used also, but it did not prove so satisfactory. Sections were cut usually at 10 μ. In large specimens, in which the mandible was quite heavy and ossified, the whole bone was removed with a razor blade or a very sharp scalpel. This method proved more satisfactory than that of decalcification of the bone. When material had been preserved for some time, Mayer's haematoxylin with eosin as a counterstain was used occasionally, but the details of the cells showed poorly. Fresh material was fixed either in picro-aceto-formol, Flemming's fluid, Vom Rath's fluid, mercuric chloride, Zenker's or Helly's fluids. Of these, picro-aceto-formol, Zenker's and Helly's fluids seemed to fix the tissue desired so that the clearest and best staining would result.

In addition to the material obtained from normal specimens, regions of the oral cavity were removed from thyroid-fed tadpoles of Bufo. The latter were preserved whole either in Helly's or Flemming's fluids and their lower jaws were not removed until the tissue was quite hard, so that there would be as little scraping off of tissue as possible. The floor of the mouth of each was sectioned.

### *Method of thyroid feeding*

The method of thyroid feeding used was similar to that employed by Gudernatsch ('14). Tadpoles were put into flat glass dishes, ten to a dish, with a control set to every experimental one. Groups at different stages of development were fed and watched in these dishes. Both the desiccated thyroid and sliced fresh thyroid from pig were used. The fresh thyroid was dried in the electric oven for twenty-four hours. The desiccated thyroid seemed to be most desired by the tadpoles when a paste was prepared from it. The paste was dried in sheets. This material was then fed to the tadpoles as if it were fish food. Three feedings of the thyroid, each feeding given every other day, was the method that seemed to cause the least sudden and profound change in the tadpoles, so that they would survive for the longest period of time and still show very striking changes.

## TONSILLAR DEVELOPMENT IN THE LINGUAL REGION OF BUFO (TYPE)

### *General description*

Since the kind of tonsil made the basis of this study has not been commonly investigated, and hence is not familiar, it seems advisable to preface the matter that follows with a general description of these structures in an adult specimen of Bufo.

In the lingual region of the floor of the mouth there occurs a pair of tonsils, one on either side of the attachment of the tongue. Some idea of their gross appearance may be obtained by reference to figure 2. Each one of the pair lies just posterior to a somewhat elevated fold of mucosa which stretched from the articulation of the jaw bone medially and anteriorly to the attachment of the tongue. These structures appear to be somewhat folded, each confined to a somewhat circular fossa, out of which they protrude slightly (in preserved material). The mucous membrane about each fossa forms a more or less conspicuous ridge which limits the extent of these structures. The tongue overlaps them fairly completely.[2]

### *Occurrence at different stages*

In studying the tonsils of the lingual region in Bufo, material was used of the eastern species, B. americanus, and the western species, B. boreas. A range of individuals, from tadpoles 16 mm. in length (with only hind limbs developed) to adults 97 mm. in length, were selected. The floor of the mouth of each was removed and sections of the lingual region were examined for the presence of tonsillar material.

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[2] Kingsbury ('12) has given the name 'sublingual' tonsils to this pair. Perhaps a more suitable name could be substituted, since the tonsils in other regions might be given this name with equal fitness. Since the name has been used, however, it can be retained for the purpose of this study, realizing at the same time that it should be qualified to really describe that pair of tonsils to which it refers.

The form which was most distinctly larval, in which the developing tonsil could be recognized, was in a tadpole which had a tail stub 7½ mm. in length, and one arm had erupted. The lymphocytes had somewhat invaded the epithelium in the floor of the mouth. Paired structures of this nature were found to occur in practically all of the tadpoles from this stage up to transformation (figs. 12, 13, 14). The exact time of the appearance of each tonsil of the pair seemed to differ, depending upon the individual.

After transformation, in specimens 12 to 40 or 50 mm., every toad possessed this particular pair, the size increasing with the size of the animal. The transection of the lower jaw shown in figure 4 is that of a young adult of the stage above described. The tonsils are large and conspicuous. Figure 15 pictures the 'sublingual tonsil' of a young toad under higher magnification. Figure 16 illustrates a tonsil in an older toad.

A medium-sized toad also showed other patches of more or less complexity. Some were paired and some were not. Patches in this general region were located on the undersurface of the tongue and in small lateral folds of the mucosa of the tongue between the expanded portions of this structure and the floor of the mouth. The general aspect of these more variable and inconstant tonsillar patches is shown in figures 6, 7, 8, and 11.

In the case of the largest toad examined, that is, a 97-mm. adult, lymphocyte accumulations were encountered in great numbers in the undersurface of the tongue. Some of these accumulations were merely little clumps of lymphocytes, seeming to be entirely limited to the epithelium. The main large pair of tonsils of which the writer has made mention in younger toads proved to be very extensive and formed two long strips of tonsillar material on either side of the midline under the tongue. Sections of the lingual region showing these two groups are pictured in the photographs (figs. 9 and 10).

In general, the developmental history of the sublingual tonsil is as follows: The tadpoles at the stage in which at least one arm had broken through and the glottis is opening may be expected to have some accumulation of lymphocytes at that place on either side which is the forerunner of the main tonsil. The number of lymphocytes will increase steadily (figs. 12, 13, and 14) and the tonsillar structure resulting will become larger and larger in proportion to the size of the floor of the mouth until the transformed toad reaches a length of approximately 14 to 20 mm. Subsequently, there seems to be less proliferation in the tonsil, and they become relatively smaller in proportion to the floor of the mouth. There follows accumulations of lymphocytes in other places, usually in the general region of this main pair of tonsils. Instead of a diminution in the amount of tonsillar tissue with age, such as occurs in man, there is actually an increase. In fact, in the region under the free posterior end of the tongue, there are very few places in which one will not encounter, in an old toad, lymphocytes in more or less extensive accumulations.

## MORPHOLOGY AND MORPHOGENESIS

In the individual in which the first appearance of the tonsillar material was noted, closer examination shows that the metamorphosis from the tadpole to the adult stage is evidently quite advanced. There is on either side of the septum glossohyoideum, in this region, the lingual artery, vein, and nerve grouped together dorsal to the hyoid cartilage (fig. 12) and dorsal to the small separations in the connective tissue which mark the future site of the ceratohyal lymph sinus. In the overlying epithelium and connective tissue there occurs on either side a small mass of lymphocytes, in the region just posterior to the caudal margin of the tongue. Most of the accumulation is slightly lateral to the lingual vessels and nerve. Figure 13 shows this relationship on the right side.

Capillaries are seen to be particularly numerous in the connective tissue of this region.

The lingual artery, vein, and nerve are well developed in this general location for some time previous to the beginning of the tonsil. In the earlier stages, however, they are somewhat lateral to the exact site indicated for them at the first appearance of the tonsil. The ceratohyal lymph sinus and the capillaries seem to develop as the lymphocytes accumulate.

During the period in which the tail stub is gradually lost, up to transformation, the general morphology of this region changes very little. The sinus expands horizontally to quite a degree, and vertically but slightly. The hyoid cartilage becomes much thinner and differentiates into a form which is quite similar to that of the adult. That region on either side occupied by the tonsil tends to lie relatively nearer to the midline, as do also the lingual artery, vein, and nerve (fig. 4). The epithelium becomes thickened, causing quite an upfolding. This folding of the mucosa becomes increasingly marked until the toad reaches a length of 15 to 20 mm.

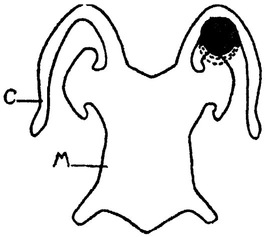

Fig. 1 Dorsal aspect of hyoid cartilage of adult Bufo. Full-lined circle, shaded, indicates position of sublingual tonsil of average size. Dotted lines indicate extent of tonsil in much larger forms (e.g., Bufo boreas). *C*, cornu principale of hyoid; *M*, manubrium of hyoid.

At approximately the size just mentioned, the tonsil has the relation to the hyoid indicated in the accompanying sketch (fig. 1). It lies dorsal to the region just posterior to the loop of the cornu principale (Gaupp, '99), and extends caudad over the processus alaris. The tongue, in its growth, has extended back, quite covering the tonsillar region.

In toads of medium size and slightly larger the mucosa over the whole surface of the tongue is thrown into folds as figured (fig. 4), and when the tongue is not extended, or when the muscles in this region are contracted, the tissue around the tonsillar area is ridged about the latter as if the lymphatic tissue of each side were in a shallow fossa. This area seems to become more elongated with the further development of the tonsil. This elongation takes place almost entirely in a caudal direction. When the floor of the mouth is quite contracted and the tongue is not extended, the elongation is not so apparent.

It is noteworthy also that the epithelium becomes increasingly thick and infiltrated to a greater degree with lymphocytes, while the underlying connective tissue retains comparatively few. No definite connective-tissue capsule is formed.

As to the vascular element in the morphology of the tonsil, there is some change after late transformation and the early adult stage. In the young forms there are several venules which drain into the lingual vein, but in the young adult toad (fig. 4) a small vein has developed to drain the tonsil, and this has become quite a definite structure in a medium-sized toad (figs. 5 and 15). The time of the definite appearance of this vein seems to be a variable thing, however, for in a toad 15 mm. in length and in one 21½ mm. this vein is not recognizable.

Another modification which occurs is the gradual formation of a fold under the tongue, in which the hyoid cartilage is found (compare figs. 9, 10). This fold seems to be simply a lifting up of the floor of the mouth, and an infolding on either side under the cartilage. In general, tonsillar tissue seems to be present in largest amount in this fold, but is not entirely limited to it.

The floor of the mouth of the very largest toad (97 mm.) which was examined showed a great lateral extension in the area occupied by tonsillar material. The mucosa is much folded, and several veins are present to drain the main sublingual tonsil (figs. 9 and 10).

## HISTOLOGY AND HISTOGENESIS

This will only be considered with reference to the main pair of tonsils, the 'sublingual' ones. The other variable tonsillar patches appear considerably after transformation, but there is no reason to consider their histogenesis fundamentally different in any essential feature.

The study of the histogenesis of this region must begin at a little earlier stage than its morphogenesis. The histogenesis might be considered to begin at the time when lymphocyte formation commences to be somewhat localized in the region which will later be the site of the tonsils. Such a condition is noted in a tadpole with a tail 14 mm. long and without the eruption of either fore leg. Before this stage, the connective tissue in practically the whole jaw appears to possess a slight leukocyte-forming activity, and no one area produces more than any other.

It is noteworthy that the observations made upon this tonsillar region, from the very earliest stages to the adult, seemed to confirm quite definitely Hammar's interpretation that the lymphocytic cells of the tonsil are differentiated from the mesenchyme in that region. In the first place, the intermediate stages, from a connective-tissue (or mesenchymal) cell to a lymphocyte, are quite clearly and distinctly recognizable in the very young form. In the second place, there seems to be no evidence that they could have come from the epithelium, since these cells occur in other places in the connective tissue far from the epithelium. As for the remaining possibility, that of the infiltration of these cells into the connective tissue from the vascular system (Stöhr, '90), all the evidence seems to show that the lymphocytes are carried away from the region in venules, and not brought in by arterioles. Kingsbury ('12) noted venules loaded with lymphocytes leaving the tonsillar region; and the writer has observed the same thing upon examining the lingual tonsils of toads of all ages.

In the very young tadpole, before the appearance of any definite tonsillar structures, comparatively few lymphocytes are present; in fact, there are slightly more leukocytes with irregularly shaped nuclei than lymphocytes. Both types of leukocytes are found in the venules. Both seem to be differentiated from the connective-tissue cells, but not from cells of the same size. An intermediate stage in the formation of these two kinds of cells can be recognized. Occasionally one may find a large cell with a somewhat irregular form, and with a fairly clear, large nucleus, that could be considered a 'lymphoid hemoblast' or 'lymphoblast' (Naigeli).[3]

In this early stage the ceratohyal lymph sinus is just beginning to appear in the very loose connective tissue as several separate spaces. The connective tissue itself shows an ever-increasing tendency to form fibers diagonally from the lateral edge of the hyoid cartilage to the grouping of the lingual artery, vein, and nerve. The appearance, later on, is similar to strings being stretched from one to the other place of attachment (fig. 12). The more pronounced this tendency, the more lymphocytes seem to be accumulated in its meshes. The epithelium is flattened and shows a slight tendency to stratification in the tonsillar region. The tissues seem to be in a particularly plastic state, probably due to the extreme changes and adjustments in morphology which are just being completed. Little groups of epithelial cells are being elevated to project into the oral cavity, and other groups sink in toward the connective tissue apparently to be absorbed later. Capillaries are few in number at this particular stage and yet seem more than adequate for the small region they drain. Possibly the presence of fairly large numbers of lymphocytes activates the formation of these tiny vessels through which later many free 'wandering cells' are carried away.

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[3] The term 'lymphoid hemoblast,' suggested by Danchakoff, refers to a large wandering cell, which has an immense, clear nucleus and rises from the connective tissue. It can potentially form any type of blood cell, according to the monophyletic theory of blood formation. Although the description of such a cell corresponds to the kind which the writer has distinguished in the region of the lingual tonsil it seems to differentiate into leukocytes, with the occasional formation of eosinophiles. Perhaps 'leukoblast' would be a better name; but implying the limitations mentioned above, the term 'lymphoid hemoblast' will be used.

With the progressing metamorphosis of the tadpoles, further changes occur in the tissues of the tonsillar region. The connective tissue becomes more fibrous, but still does not for some time lose its capacity to differentiate leukocytes of both types. The amount of connective tissue lessens markedly, and apparently there is considerable pressure upon it in the region about the lingual arteries, veins, and nerves for these suggested reasons: 1) the ceratohyal lymph sinus expands in all directions; 2) the epithelium is thickening; 3) there is a strong tendency to lessen the thickness from the dorsal to the ventral surface of the floor of the mouth, and, 4) the hyoid cartilage expands somewhat, laterally.

Beside the factor of pressure, there seems to be, in addition, that of a pull or strain of the connective tissue in this region, undoubtedly due to the extensive lateral growth of the hyoid cartilage, and the more medial shifting of the lingual artery, vein, and nerve, producing a greater length between these two points. It seems plausible that this would tend to cause the connective tissue to become fibrous and more condensed.

The leukocytes described previously, which appear to be the 'polymorphonuclear' type, become less obvious. There is a range in the size of the lymphocytes, from those ordinarily classed as 'small' (Jordan, '19) to those called 'large.' These cell types increase in number considerably, and form the majority of the leukocytes in the tonsillar region.

By the time that the tails of the tadpoles have decreased to 8½ mm. in length, the epithelium has become cuboidal. When one arm has appeared and the tail stub has reduced to 7½ mm., the epithelium has become stratified in some regions, particularly in the one of interest in this study. Lymphocytes have begun to infiltrate the epithelium. Their formation remains fairly general in the connective tissue underlying this epithelium of the oral region.

At transformation, the epithelium in the region surrounding the tonsil has gained cilia, and the outer layer is of the columnar type. At the site of the tonsil, very few cilia are developed, and the epithelium may be considered to be stratified columnar or cuboidal. The ceratohyal lymph sinus at this stage is practically always completely formed, and the connective tissue above it has become more fibrous.

From this stage to the adult, the epithelium becomes more and more thickened and changes markedly wherever it is greatly infiltrated with lymphocytes. In the tonsillar area the cells of the surface layer lose their columnar or cuboidal character, becoming flattened, and the cilia are lost. The deep layers of the epithelium become somewhat reticulated.

Capillaries are numerous in the tonsils. Little strands of connective tissue extend up into the epithelium in a manner somewhat similar to the connective-tissue papillae in the skin. In this case, however, the strands are so delicate and thread-like, that the general impression is given that the capillaries actually penetrate the epithelium.

The connective-tissue elsewhere in the tonsil becomes more and more vascular, and contains within its meshes large numbers of lymphocytes, especially nearest the epithelium. It is altered very little, however, from that in the neighborhood of the tonsil, and is in contrast to the condition found in mammals.

In the late transformation and adult stages the smaller lymphocytes are in the majority, but the larger-sized ones are present in considerable numbers. Occasionally lymphoid hemoblasts may be distinguished. Large cells, possibly early stages of hemoblasts, may be found, particularly about blood vessels. It should be mentioned also that, especially in the small inconstantly occurring tonsillar patches, the lymphocytes seem to be grouped about blood vessels, with very few such cells in the connective-tissue about this area. Not uncommonly, eosinophiles may be found present as in figure 17. Lymphocytes in mitosis can be noted at practically all stages. They are particularly abundant in the tonsils of toads shortly after transformation, though readily found in the tonsils of toads as large as 67 mm. in length. The lymphocytes and lymphoid hemoblasts are quite like those found in the higher vertebrates and like those described from the blood of a frog by Jordan ('19). In early stages it would seem that all the lymphocytes develop from hemoblasts, but in older forms there seems to be proliferation of the 'large' lymphocytes, as well as formation of lymphocytes from hemoblasts derived in small numbers from the connective tissue.

## DEVELOPMENTAL ORIGIN

The solution of the question as to the developmental transformations underlying the origin of the 'sublingual' tonsils offers some difficulties. At least half of the period of metamorphosis is passed through before the appearance of the tonsil, and during this period quite profound changes are undergone, especially in the oropharyngeal region. Kingsbury ('12, p. 606) states that "The sublingual tonsils appear . . . . at the cephalic end of the furrow II leading forward from the branchial chambers." The site of the tonsil can be traced back to quite an early stage by following its associated structures, the lingual artery, vein, and nerve and the broadest part of the hyoid cartilage. The region with which this study is concerned is considerably elevated previous to the appearance of a localized area of leukocyte formation. As nearly as the writer can ascertain, without the investigation of any material of tadpoles younger than the one with a tail of about 17 mm., these prominences on either side are at the cephalic end of the furrow II. It is in the last stages of the flattening out of these prominences, which are obviously remnants of pillars between the first and second pouches, that the tonsils appear.

There are two marked sets of adjustments that are made then. First, there is the reducing of these villiform prominences to fairly flat surfaces. This involves a 'coupling up' of the epithelium, which probably increases its tendencies later to be thickened and infiltrated with lymphocytes. Also there is an excess of connective tissue temporarily.

The second adjustment is one which tends to pull out or stretch this connective tissue in a manner described in the discussion of the histogenesis of the tonsil.

## EFFECT OF THYROID FEEDING

An interesting phase of the study, which was merely begun and needs more thorough work, was that made upon toad tadpoles which were induced to transform prematurely by thyroid feeding. The tadpoles treated in this fashion transformed before their controls had even gained their fore limbs.

The lingual region of some tadpoles that were thyroid fed were sectioned. Individuals at the stage just before transformation and at that period were chosen. It may be noted that the tadpoles were preserved just after they had died, and it is possible that there might have been some alteration on that account. It was found that, although the small toads assumed the general gross form of normal transformed toads, histological study showed that the resemblance went practically no further. There was very little cellular differentiation. The hyoid cartilage was practically the only structure that was fairly well differentiated. In those regions, such as the tongue and the dorsal part of the lower jaw, where a great deal of change occurs normally during metamorphosis, the epithelium appeared single-layered, or sometimes a tendency to several layers was noticeable. The muscular tissue was very poorly formed in all cases, and sometimes was hardly recognizable as such. The lingual artery, vein, and nerve were present, although often the artery and vein were nothing more than single-layered endothelial tubes. The interesting feature from the standpoint of this paper is that no tonsils were developed. In many cases cells that appeared to be similar to lymphocytes, or hemoblasts, were present in the connective tissue, quite generally distributed, but the differentiation of the connective tissues in the region of the lingual artery, vein, and nerve above the body of the hyoid cartilage did not take place. A further study of thyroid-fed tadpoles at these stages might help to clear up some of the details of tonsil formation, if the tadpoles could be induced to live after transformation.

## TONSILS IN REPRESENTATIVES OF OTHER ANURAN FAMILIES

Part of the present study consists of an investigation of the occurrence and character of tonsils in the lingual region in other families of Anura. This is more or less of a comparative study, and a compilation of the results is presented in the appended table.

### *Ranidae*

There exists such a difference in the species of the representative genus Rana that it seemed advisable to examine three different ones. As mentioned previously, individuals before transformation at that stage, just after transformation, and in the adult stage were chosen, and the floor of the mouth of each was removed for study.

*Rana catesbiana.* In this form the occurrence of tonsillar material is a little different from any other anuran species that was examined, because of the characteristic presence of tonsillar tissue in the mucosa of the dorsal part of the tongue. The tonsillar patches are not restricted to the upper surface of the tongue, for there are some smaller accumulations of lymphocytes on the floor of the mouth under the tongue which are quite like those found in other species of Rana that will be described.

Tonsillar material on the dorsal surface of the tongue does not seem to increase in amount with age. This type of tissue may become quite localized in this region in an old bullfrog.

The structure of the small masses of tonsillar tissue on the surface and sides of the tongue in younger adults and those just after transformation have a crypt-like form. The whole surface of the tongue of the young bullfrog is thrown into folds, and the apex of each fold is expanded to give a mushroom-like appearance. It is in the epithelium of the crypts formed by these folds that the lymphocytes accumulate. The epithelium is thickened and its character is changed radically. No cilia are present upon the epithelial cells of the tonsil where the latter is quite a definite structure.

The tongue of this form is fairly muscular, and the connective tissue is limited in amount. Being of a rather dense, fibrous nature, it forms a rudimentary inner limit to the tonsils, without being specialized for that function.

In other places in the lingual region where accumulations occur, the vase form is not found. The epithelium contains the most of the lymphocyte accumulation and may be so thickened as to form a prominence. In these places the connective tissue is altered slightly. It is not so fibrous, but is more reticular. The lymphocytes vary in size in the tonsil as they do in the blood, and they are quite round.

The noteworthy points concerning the tonsillar tissue in the lingual region of R. catesbiana are these: 1) tonsils appear after transformation in the young adult; 2) they may occur on the floor under the tongue, on the inferior or lateral surface of the tongue, and do occur characteristically in the dorsal surface of this structure. But their exact location seems to be inconstant and they are not paired. On the dorsal surface of the tongue, the accumulations usurp the crypts of glands, apparently, and change their aspect entirely (fig. 21). The vascular supply is always abundant, but a nerve supply cannot be traced.

It is of interest to note the occurrence of a small accumulation of lymphocytes in the skin on the ventral surface of the head, underlying the floor of the mouth. It is evidently related to a small gland at that spot.

*Rana sylvatica.* Individuals of this species were examined, but in none of the stages did the author detect any well-organized, definite, and constantly occurring tonsils. Small tonsillar patches, inconstant in position and quite diffuse in character, were to be found anywhere on the lateral or ventral surfaces of the tongue or in the floor of the mouth immediately under it (fig. 19). Such patches are not found until just after transformation.

As to the histological character of these structures, it may be noted that the epithelium, which is typically ciliated and pseudostratified with frequent mucous gland cells, becomes somewhat reticulated and thickened, losing most or all of its cilia. These epithelial cells, in general, are very large in proportion to the other epithelial cells in the floor of the mouth and to epithelial cells in other forms of anura. In the loose and reticulated connective tissue it is possible to see stages of cells from a slightly changed connective-tissue cell to a lymphocyte in one section. The writer did not note any mitotic figures among the lymphocytes and concludes that perhaps they arise from the poorly differentiated connective tissue. There is always a good vascular supply in the connective tissue at the site of the tonsils.

*Rana pipiens and R. palustris.* These two forms are so similar in respect to the occurrence and character of their tonsils that the writer can conveniently discuss them together. The tonsils are better developed in these two species than they were found to be in Rana sylvatica. They become much denser structures, since the accumulation of lymphocytes is greater in amount, although here, too, they are inconstant and not paired.

Tonsils in the lingual region are found to occur on the ventral surface of the tongue, on the floor of the mouth under the tongue, usually where the latter is free and in the tissue which joins the tongue to the floor of the mouth.

In this form, also, the tonsils do not appear until after transformation. They appear as dense little prominences or hillocks as figured (figs. 18 and 20).

The epithelium of the tonsils is somewhat like that encountered in R. sylvatica. The connective tissue is more fibrous than that found in the latter form, but not so fibrous as in R. catesbiana. The vascular supply next to the epithelium everywhere in the lingual region is very extensive.

The lymphocytes themselves are of the type described by Jordan ('19) as occurring in the blood of R. pipiens. There are all gradations from small to large lymphocytes, and occasionally one sees a very large cell with a fairly clear nucleus, which might be classed as a lymphoid hemoblast.

### *Hylidae*

*Hyla crucifer and Hyla versicolor.* These two species were selected as representative of this family. They are very similar in appearance, and an histological study of them showed that they were quite similar in their minute structure. In neither species could the writer locate one tonsillar patch at any stage. The amount of connective tissue is not great anywhere, and the peculiar epithelioconnective-tissue relations (and perhaps vascular relations) which seem to underlie the tonsil formation in the other anurans apparently do not exist here.

Since the Hylidae are quite closely related in some respects to the Bufonidae, it was rather to be expected that the tonsillar tissue might be developed also in Hyla. The general appearance of the tongue region is quite similar, and what little connective tissue there is, seems much like that found in Bufo. It should be added, however, that lymphocytes do occur diffusely in the connective tissue about the muscles in the region of the tongue and therefore these cells can be differentiated in this form.

### *Discoglossidae*

*Ascaphus truei.* This group is also very closely related to the family Bufonidae. The tongue in this form, however, is very nearly immovable, and the type of stress and strain set up, consequently, might differ sufficiently to account for the fact that the occurrence and character of the tonsils found in the two groups are not the same.

No tonsillar tissue could be distinguished in individuals that were in the stages through transformation. But in the form just after transformation, quite a few small accumulations were noted. At the following sites tonsils were recognized: 1) in the median line in the floor of the mouth above the broadest expanse of the hyoid; 2) in a situation on one side comparable to the position of the sublingual tonsils in the toad (fig. 26); 3) a little inpocketing under the floor;

4) small inconstant ones in the floor under the tongue; 5) and an occasional one on the upper surface of the tongue.

The connective tissue is quite altered from its wavy, fibrous character, at the site of the tonsils, and a reticular network is produced. The condition which Latta ('21) describes as occurring in the epithelium over Peyer's patches in the rabbit seems to occur in the connective tissue of Ascaphus truei. Around each little lymphocyte a clear space appears which can hardly be considered an artifact. The epithelium is less altered in the tonsil here than in any other form used in this study. The mucous cells persist to quite a large extent.

An occasional site of accumulations of lymphocytes occurs often about and in the connective tissue of the muscle bundles in the floor of the mouth in this form. This was also found in the toad. Although this fact is not altogether relevant to this study, the writer feels that it might be of general interest in connection with the problem of the lymphocyte.

### *Pelobatidae*

*Scaphiopus hammondi and Scaphiopus holbrooki.* These two species were used as representatives of this family. In these forms it was found that no tonsils were developed until after transformation.

In the adult they are fairly well developed as compared with such structures in other amphibia. They can be found upon the floor of the mouth in the lingual region and on the under and lateral surfaces of the tongue (fig. 25). The lateral lingual tonsils found in a 58-mm. individual (figs. 23 and 24) were best developed and were paired structures. The pair which is of most interest, however, is the one which occurs in both the young and old adults examined, and can be considered a sublingual tonsil, although it is far less developed than that of the Bufo.

Histologically, the structure is quite like that observed in other anurans, with the exception of the lymphocytes. These cells are very small. Possibly this is a generic character.

The floor of the mouth of the adult, particularly, resembles that of the toad very much. The fold under the hyoid cartilage, the size of the ceratohyal sinus, the position of the lingual artery, vein, and nerve, are all very similar. The tongue, however, is slightly different, tending to be bifid at the posterior margin.

### *Brevicipitidae*

*Gastrophyne caroliniensis.* This family of so-called 'small-mouthed' toads is, according to the systematists, perhaps least closely related to the Bufonidae of any of the groups commonly classed as 'toads.' However, from the standpoint of the structure of the floor of the mouth and occurrence of the tonsils, the members of these families would be considered as quite closely related. It is obvious, however, as will appear on comparison of the tonsillar relations in the different anura, that the tonsils are not trustworthy as structures of taxonomic value.

It is noteworthy that no tonsils can be found until the toad is a full-grown adult. Generally speaking, Gastrophyne has tonsillar patches only under the tongue, over the hyoid bone, and on the floor of the mouth. Paired ones were found in these locations: 1) one on either side lying dorsal to the connective-tissue septum which joins the manubrium and cornu principale of the hyoid cartilage; 2) on the dorsal border of the lower jaw about at the junction of the squamous and columnar epithelium in the region where the tongue is attached to the floor. In one case there was an accumulation of lymphocytes in the epithelium of a gland on the surface of the tongue. The lymphocytes here were associated with almost an equal number of eosinophiles, and the presence of the latter would seem to indicate that this occurrence was caused by some unusual conditions.

The pair of tonsils mentioned first is situated quite like the sublingual tonsils in the toad, but are considerably less developed (fig. 22).

*Table of lymphocyte accumulations in representative Anura (except Bufo)*

| FORM | OCCURRENCE | LOCATION | CONSTANCY |
|---|---|---|---|
| **Ranidae:**<br>**Rana catesbiana** | + | Typical—on surface of tongue<br>Other locations: 1) Under surface of tongue. 2) Floor of mouth under and lateral to jaw | Inconstant except in general location |
| **Rana sylvatica** | + —<br>Somewhat inconstant in general occurrence | Typical—*a*) ventral or lateral surfaces of tongue; *b*) dorsal surface of the floor of the mouth immediately under the tongue<br>Other locations: dorsal surface of tongue | Inconstant except in general location |
| **Rana pipiens and palustris (very similar)** | + | Typical—*a*) ventral surface of tongue; *b*) on the floor of the mouth under the tongue; *c*) between the floor and under surface of the tongue | Very inconstant |
| **Hylidae:**<br>**Hyla versicolor and Hyla crucifer** | — |  | Lacking |
| **Discoglossidae:**<br>**Ascaphus truei** | + | Typical—*a*) on the floor of the mouth and in pocketing of the floor; *b*) under surface of the tongue<br>Other places: upper surface of the tongue | Not enough individuals to state definitely |
| **Pelobatidae:**<br>**Scaphiopus hammondi holbrooki** | + | Typical—*a*) floor of mouth quite like B; *b*) under surface of tongue; *c*) lateral surface of tongue | Small pair of sublingual tonsils (less developed than in Bufo) — are probably constant |
| **Brevicipitidae:**<br>**Gastrophyne caroliniensis** | + | Typical—1) pair of tonsils quite similar to the sublingual ones in Bufo; 2) over jawbone in region where tongue is attached; 3) accumulation above corpus hyoid<br>Other places: on dorsal surface of tongue | Probably sublingual pair is fairly constant |

## DISCUSSION

### *Causes of localized lymphocyte formation*

There still remains one phase of the consideration of the tonsil which has been touched upon but slightly. This is the problem of the most common and characteristic cell of the tonsil—the lymphocyte. The derivation of this cell has been considered, and it was concluded that it was formed from the connective tissue. The conditions found where the accumulation of lymphocytes arise have been described, while the probable factors behind these conditions were suggested. The determination of such factors is clearly of marked importance for understanding the lymphocyte. Why this cell form is produced in such numbers in these particular localities is a question of primary interest.

Some factor of stress or 'strain' has been suggested previously in this paper as the possible answer to this question, when the developmental origin of the sublingual tonsils were discussed. This same factor was suggested by Rand ('19) to be associated with the lymphocyte formation in the pharyngeal tonsil of the calf. The changes which take place during the period of metamorphosis and which create the conditions which cause lymphocytes to be differentiated have been earlier enumerated.

After transformation, it is noted that the sublingual tonsils persist at the point of origin after the factors which probably give rise to their formation have disappeared. It would naturally be expected that there would be a gradual disappearance of these structures as the adjustments were made. The explanation not only for their persistence, but for their further growth, is to be sought. The possibility suggests itself that a secondary set of strains arises, as the tongue, particularly, develops and functions in a somewhat different manner. Reference to figures 2, 3, and 4 shows that, in the adult, this main pair of tonsils is located, one tonsil on either side, at a point where there is a lateral pull—due to the attachment of the tongue, and an anteroposterior pull from a point just cephalad of the tonsils, backward. This might be a secondary locus of strain. It should be recalled, also, that the toad extends its tongue so that it is thrown far forward, when the prey is captured. The extension and retraction of the tongue at the point described, would exaggerate the strain considerably. That this is really the case is a difficult point to prove, but the evidence appears strong that these conditions, at least in general, may explain the persistence of the sublingual tonsils.

The conclusion which the writer draws, then, is that the site of the accumulation of lymphocytes is determined largely, if not wholly, by purely mechanical factors arising during the period of metamorphosis or afterward, and that the persistence may be comprehended on the same basis.

### *Relation of lymphocyte to epithelium*

The great infiltration of lymphocytes into the epithelium is a second characteristic of the tonsil demanding an explanation. In Amphibia the close relationship of these lymphocyte cells to the epithelium is marked. Infiltration of lymphocytes into this surface tissue occurs in the tonsils of mammals to some extent, but in amphibia the epithelium in the tonsillar region becomes so crowded with these cells (compare figs. 6, 15, 16, 25) that, in some cases, as in the inconstant and later developed tonsils of the toad, the majority of the lymphocytes are present in this tissue, and not in underlying connective tissue. The reason for the migration of the lymphocytes into the epithelium is difficult to ascertain. Two aspects (or possible explanations) of the question present themselves: is there some character or quality of the epithelium at the general or specific sites of accumulations which induces the infiltration of lymphocytes into it; or is the case rather that the epithelium is more or less passive, and the migration of lymphocytes into this surface tissue is caused by factors affecting or active in the underlying tissue?

Concerning the first suggestion, one must consider the several lines of evidence which seem to support the idea that infiltration is induced by some character of the invaded tissue. The question at once arises as to whether these structures are limited entirely to the epithelium of endodermal origin. This suggestion may be discarded immediately when it is recalled that, in at least one case, R. catesbiana, there was noted in this investigation a lympho-epithelial accumulation associated with the skin—an epithelium of ectodermal origin. Jolly ('19) similarly described a 'lympho-epithelial mass' in an ectodermal location, as was noted on page 404. It follows, then, that the germ layer does not determine, even in a general way, the tonsil-forming potencies of the surface tissue involved. Far more difficult of definite answer is the question as to whether there is any characteristic of the epithelium at the specific sites affected which determines the lymphocyte infiltration. Perhaps the structure of this tissue is different at these particular sites, due to causes arising from developmental changes or to conditions arising in the adult stages. There is a suggestion that this may be the case in the early development of the sublingual tonsils of the toad. At that period there are profound readjustments made in the surface tissue to effect a flattening out of prominences. At the site of the future tonsil there is a noticeable tendency for the epithelium to thicken considerably. Fairly similar thickenings occur, however, in other places in the general region of the floor of the mouth also, and the significance of the particular thickenings at the site of the tonsil is hard to estimate. That the epithelial tissue has any different structure at the site of the tonsils of the more variable and inconstant type is difficult to demonstrate, since it is impossible to trace back the conditions which preceded the infiltration of the lymphocytes. From the large numbers of sites at which accumulations of these cells are found (for example, in the lingual region in the adult toad), it would be necessary to conclude that there are great numbers of places in this tissue which in some way differ in their characteristics, so that lymphocytes migrate there. It would seem to the writer more probable that the epithelium has no determinative power responsible for lymphocyte accumulations or for its infiltration.

Attention may be called to the relation of glands and mucous gland cells of the epithelium to lymphocyte infiltration. In all anurans tonsils are found at sites where the surrounding mucosa contains glands. Often a few cells of the latter type will remain fairly unaltered after the lower or surrounding epithelial cells will have become radically changed by infiltration of these wandering cells. One never finds tonsillar structures in a region where there are no glands. They seem to be always associated. On the other hand, there seems to be no evidence of a close relation between the mucous secreting cells and lymphocyte accumulation. The 'sublingual' tonsil in toads is a fairly definite structure before mucous secreting cells can be recognized in the neighboring epithelium. The condition would seem to be, rather, that the characters which apparently are in a general way suitable for lymphocyte accumulation are also suitable for gland formation, and that the existence of the two resulting specialized structures are not interrelated any more intimately.

The conclusion is reached, therefore, that the epithelium is practically passive in tonsil formation. The infiltration of lymphocytes into the epithelium must be due, then, to the factors affecting, or active in, the underlying tissue. Judging from the observations made upon the material used in this study, there are several conditions present which seem to offer adequate explanation for the migration of lymphocytes into the epithelium. In the first place, the vascular supply is particularly abundant in the region of the connective tissue which immediately underlies the epithelium so infiltrated. The lymphocytes have a close relationship to the vascular supply, the importance of which will be discussed further. Hence these cells are found all about the capillaries, in the overlying epithelium, as well as in the surrounding connective tissue. In the second place, the strains and stresses which, according to my interpretation, stimulate the formation of these lymphocytes have little effect upon the epithelium, since the latter is much folded and plastic, allowing for alteration under tension. On the other hand, there seems to be no such provision for taking care of strain in the connective tissue so that these 'wandering cells' migrate into the region of less strain. Probably there is some propulsion exerted by the connective tissue, pushing the lymphocytes outward.

And, lastly, the movements of the lymphocytes in this direction do not seem to be checked materially, since there exists no membrane or limiting structure beneath the epithelium.

It seems, however, quite obvious that no one of these conditions or factors, in itself, could adequately explain the enormous infiltration of the lymphocytes into the epithelium.

### *Function of the tonsillar tissue*

As to the puzzling question of why the lymphocytes are present, there are the same possible explanations to be considered in the amphibia that there are in the other groups where tonsils exist. The fact has already been mentioned that the capillaries leading to venules are crowded with lymphocytes, and this strongly indicates that these cells are added to the blood stream. The capillaries are numerous and evidently adequate for the transportation of these leucocytic cells to the venules and veins. Thus it can be safely concluded that the tonsils are sites of lymphopoiesis. Mitotic figures are frequent, as pointed out in the discussion of the histogenesis of the sublingual tonsil.

Whether the lymphocytes have a function in situ is another question to be considered. There is no evidence, so far as the writer's investigations have shown, that such is the case. There is no indication that the cells are phagocytic, nor that they have a protective function. None is found to be penetrating the outer layer of epithelium to be added to the salivary secretions of the oral cavity. As far as can be ascertained, they are only added to the lymphocytes of the general circulation. What their significance there may be is a problem quite apart.

## GENERAL CONSIDERATIONS

### *Relation of tonsils to other similar structures*

In this investigation a very simple kind of 'lympho-epithelial' (Jolly, '23) structure is the object of study. Because it conforms to the essential requirements of a tonsil, according to the definition given in the introduction, it has been designated by that name. In tonsillar structures of as little complexity as these, however, it becomes very obvious that it is difficult to distinguish sharply between other lympho-epithelial structures and 'tonsils.' Comparing them in detail, one may ask if there are any real differences between structures such as the 'sublingual' tonsil of the toad and the thymic placode of the teleost (or even Petromyzon, perhaps[4]); or, if there is any fundamental difference between these tonsillar structures and the Kiemenreste, or the thymus of anura themselves, except the detachment of these latter structures from the surface. In the case of tonsils and the thymus, at least in these lower forms (and very likely the Kiemenreste as well), the components are epithelium, connective tissue, and lymphocytes, and the ultimate position and relationship alone really distinguish them. The structures under discussion in this paper are called 'tonsils,' realizing at the same time that, at least, the 'sublingual' tonsils, on the basis of their similarity of composition and structure, might as justifiably be grouped with the thymic placodes. Their difference in place of origin from the branchial chamber alone gives a basis for grouping them separately.

### *Normal or abnormal structures?*

Leucocytic accumulations are so frequently associated with inflammatory foci that it may be suggested by some that they are induced entirely by pathogenic conditions, and are not of normal occurrence. Such an explanation is clearly inadequate and untenable for the sublingual tonsils, since, as already shown, they are constant in a number of forms. Their early development begins at quite a definite time and in a definite location from definite embryological structures. In the case of the more variable and inconstant tonsillar patches, however, this explanation might, as a first impression, seem to be justifiable. It would be expected, however, that, if this one factor causes the accumulation of lymphocytes, some individuals would not have any accumulations, while others would vary radically in the number of patches. Such is not the case. Although the location and size of patches differ, there is not a great variation in total amount of tonsillar tissue in toads of the same age. In adults in all families of anura except Hylidae, lymphocyte accumulations are found to occur in every individual. In the one case, under the writer's observation, in which there did seem to be a suggestion of an unusual condition, possibly pathologic with an accumulation of lymphocytes, the site was located in a region different from any of the other accumulations, and a large number of eosinophiles occurred with the lymphocytes. The pathogenic explanation is thus inadequate to explain any but such accumulations as the last described.

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[4] Wallin ('17) calls these placode structures in Petromyzon 'thymus-like.' Their interpretation is, perhaps, still in doubt.

## CONCLUSIONS

1. Accumulations of lymphocytes in the connective tissue and overlying epithelium in the lingual region exist in all the families of anura except Hylidae.

2. Only in Bufo have they been found to appear before transformation is completed.

3. Lympho-epithelial structures are inconstant and variable in specific location in all forms examined except for the so-called 'sublingual' tonsils of the species of Bufo, Scaphiopus, and Gastrophyne.

4. The tonsils of anura, in general, are usually papilliform, or hillock-shaped, with an accumulation of lymphocytes in the connective tissue, and epithelium. The latter tissue is very much thickened, and greatly infiltrated with lymphocytes, while at least the lower layers are reticular. The vas-

cular supply is always abundant and has intimate association with the epithelium. There are no lymphatic nodules, no centers, and no bounding connective tissue.

5. The cells accumulated in the connective tissue and epithelium of the tonsillar region are mainly lymphocytes, grading from the 'small' to the 'large' type.

6. In all the forms it seems to hold that, instead of a decrease in the amount of tonsillar material, with age, there is actually an increase.

7. The observations made by the writer confirmed those of Kingsbury concerning the developmental origin of the 'sublingual' tonsils. They arise from or near the cephalic ventral end of pouch II, where two more or less symmetrically located elevations exist.

8. In this same form in the early stages lymphocytes arise from the connective-tissue cells (or mesenchyme).

9. It is suggested that the differentiation of lymphocytes is probably due in some way to a factor of stress and strain, arising through adjustments during metamorphosis. A secondary set of strains is probably produced after transformation, which accounts for the persistence of the tonsil. The reason for the differentiation from the connective tissue of lymphocytes, rather than some other type of cell, is, however, not clear.

10. The epithelium of a tonsillar structure seems to be more or less passive in determining the infiltration of lymphocytes into its boundaries.

11. The tonsils are a site of lymphopoiesis. The cells are carried into the general circulation through the venous system.

12. The tonsillar accumulations of the anura are very similar to the thymic placodes of the teleost (Hammar, '03) in respect to their components. Their origin and ultimate position alone differentiate them.

13. Toad tadpoles can be induced to transform prematurely without the formation of any tonsils by thyroid feeding.

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## PLATE 1

### EXPLANATION OF FIGURES

2 Bufo boreas, 80-mm. adult; dorsal aspect of the floor of the mouth with tongue removed (attachments indicated by black line). *T*, sublingual tonsil. Photo, × 1½.

3 Bufo boreas, 94-mm. adult; same as above. Photo, × 1½.

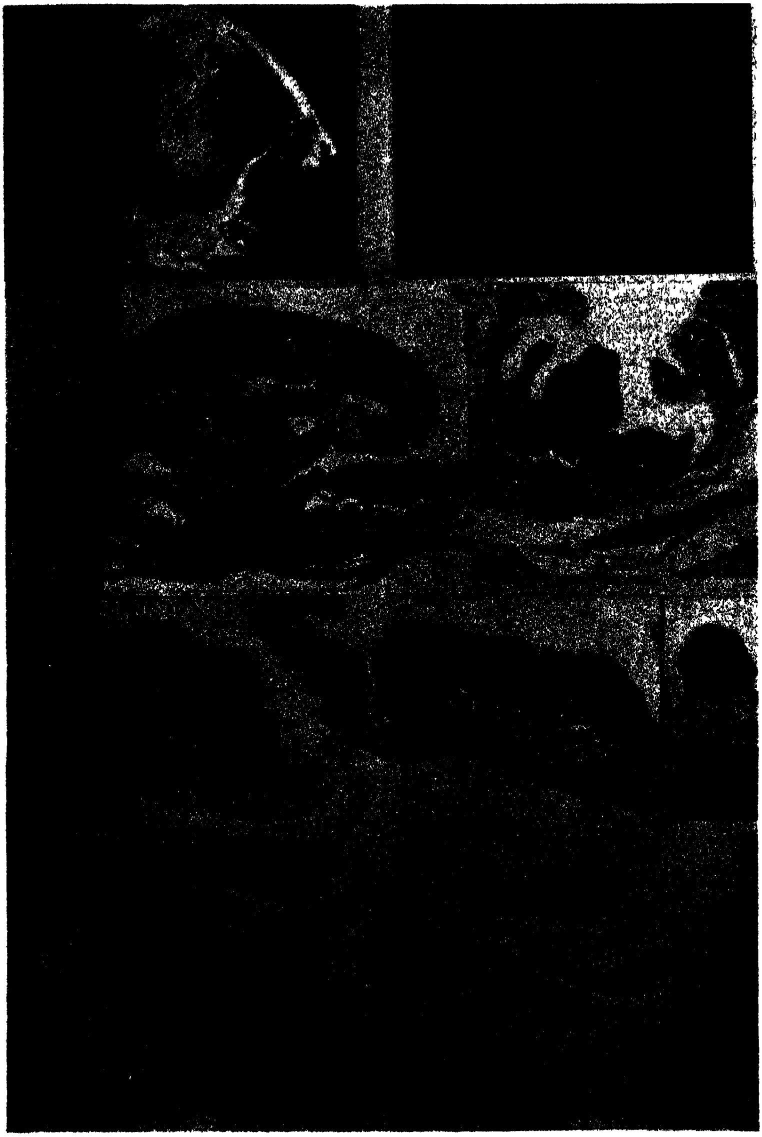
4 Bufo americanus, 11-mm. young adult; section of lower jaw through tongue. *T*, sublingual tonsil; *V.L.*, lingual vein; *S*, ceratohyal sinus; *A.L.*, lingual artery; *N.L.*, lingual nerve; *C*, cornu principale of hyoid. Photo, × 22½.

5 Sublingual tonsil of 72-mm. Bufo. Note that the tonsil exhibits something of a fossa. View of tonsil, lingual, artery, and vein. Photo, × 138¾.

6 and 7 Left and right tonsils of a pair posterior to main sublingual tonsil in a 55-mm. toad. The infiltration and thickening of the epithelium is very marked. Photo, × 138¾.

8 Small tonsillar patch located in sublingual fold. Toad, 59 mm. Note that the lymphocytes are mainly in the epithelium. Photo, × 138¾.

9 and 10 Left and right sublingual tonsils, Bufo boreas, 97 mm. *T.*, sublingual tonsil; *M.*, manubrium of hyoid; *C.*, cornu principale of hyoid. Photo, × 7½.



## PLATE 2

### EXPLANATION OF FIGURES

11 Small tonsillar patch. Bufo boreas. Photo, × 138¾.

12 Developing sublingual tonsil. Bufo americanus, transforming, with tail stump reduced to ½ mm. Some indication of stretch of connective tissue from lingual vein to hyoid cartilage. *H.C.*, hyoid cartilage. Lingual vein, nerve, and artery are on the right of the tonsillar site. Photo, × 282½.

13 Developing sublingual tonsil. Bufo americanus, transforming, tail stump reduced to ¾ mm. Note mitotic figure in the epithelium and lymphocytes around capillary. *N.L.*, lingual nerve; *A.L.*, lingual artery; *S.*, ceratohyal lymph sinus; *M.*, manubrium of hyoid. Photo, × 282½.

14 Developing sublingual tonsil. Bufo americanus, recently transformed, 12½-mm. length. Most of the lymphocytes are in the connective tissue. Photo, × 282½.

15 Sublingual tonsil. Bufo americanus. Young adult. A venule is shown leaving the tonsil (right) on its way to the lingual vein (upper left). Lingual vein, nerve, and artery are shown in an oblique row. Cornu principale of the hyoid is in the lower left. *S.*, ceratohyal lymph sinus; *T.*, part of tonsil. Photo, × 138½.

16 Sublingual tonsil. Bufo americanus, 59 mm. The epithelium is here very greatly infiltrated. Something of a tonsillar fossa exists. Photo, × 138¾.

17 Sublingual tonsil, near caudal edge. Bufo americanus, 72 mm. The lymphocytes are variable in size. Eosinophiles are present. Photo, × 282½.

18 Tonsil on under surface of tongue. Rana palustris, 53 mm. Photo, × 138¾.

19 Tonsillar patch on under surface of tongue. Rana sylvatica. Adult. Photo, × 138¾.

20 Small tonsillar patch on floor of mouth beneath tongue. Rana pipiens, 58 mm. Photo, × 138½.

11
T
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# PLATE 3

## EXPLANATION OF FIGURES

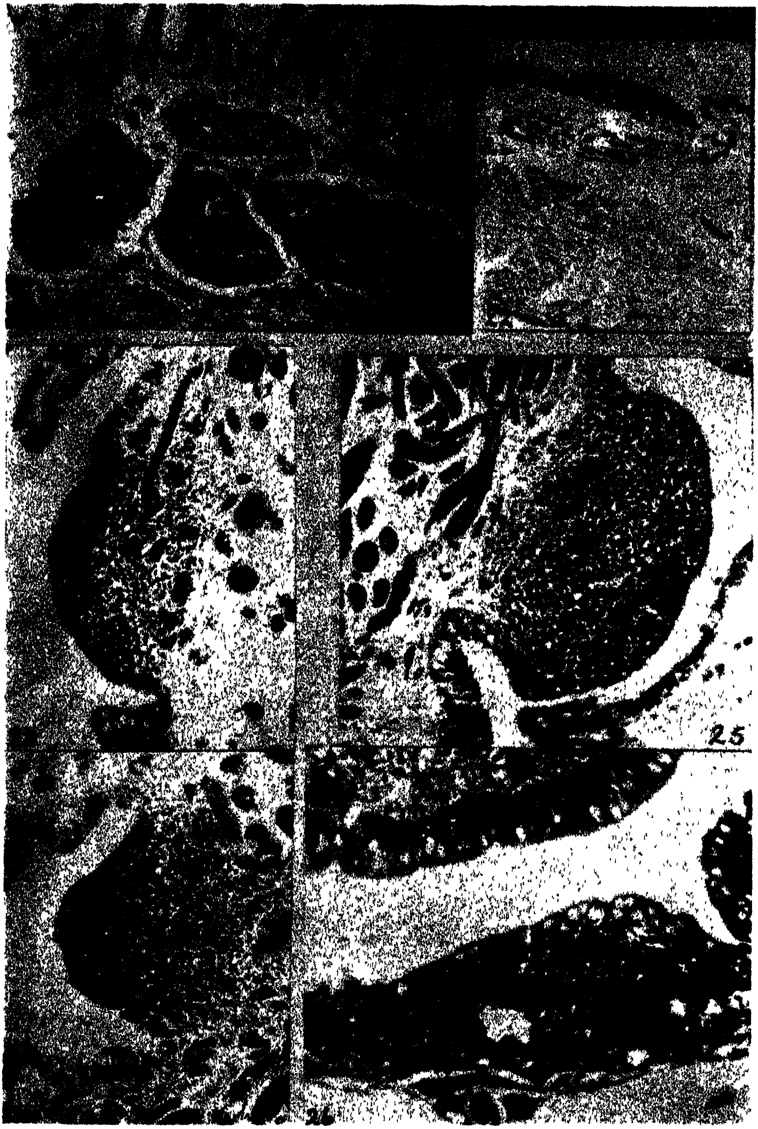
21 Lymphocytic invasion of glands, anterior to bifurcation of tongue. Rana catesbiana, large adult. Photo, × 71¼.

22 Sublingual tonsil of Gastrophyne. Adult. Photo, × 138⅔.

23 and 24 Left and right tonsils of a pair on the sides of the tongue. Scaphiopus holbrooki, 58-mm. adult. Muscle fibers are shown in close association. Photo, × 138⅔.

25 Tonsil on undersurface of tongue. Scaphiopus holbrooki, 58-mm. adult. The inner border of the infiltrated epithelium may be distinctly seen. Photo, × 138⅔.

26 Tonsil in floor of mouth. Ascaphus. 40-mm. adult. A venule is in the midst of tonsil. The epithelium is but slightly infiltrated. Photo, × 138⅔.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26

# THE SEPTOMAXILLARY

## I. IN THE AMPHIBIA URODELA[1]

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SEVEN PLATES (TWENTY FIGURES)

AUTHOR'S ABSTRACT

The septomaxillary bone is described in the various families of the urodeles in which it occurs. Its identity as a hitherto unrecognized cartilage bone of the skull is established and its close relationship with the nasal muscles followed through the group. Its presence or absence, which is found to be another criterion for the recent classification of the urodeles as proposed by Dunn and Higgins, is correlated with the development of the accessory dilatator muscle, since it occurs only in groups where this muscle attains an appreciable size.

CONTENTS

Introduction . . . . . . . . . . 441
Material and methods . . . . . . . . . . 445
Description . . . . . . . . . . 447
The nature of the septomaxillary bone . . . . . . . . . . 453
Summary . . . . . . . . . . 454
Literature cited . . . . . . . . . . 456

### INTRODUCTION

Up to the present time, the bony element of the nasal region in certain groups of the lower vertebrates, now usually described as the 'septomaxillary,' has received little or no attention from the various investigators in this field, even its nature and origin having been given controversial description. According to Gaupp ('05), "Parker nennt ihn Septomaxillare" in the frog, without comment as to its name. It was first described and named 'cornet' by Ant. Dugès ('35).[2] Cuvier (1817) figures the turbinals in the skull of 'Le Grande Python de Java,' and calls them 'les cornets inférieurs.' According to Ecker ('64),[3] he described the little bones in the skull of the frog as fixed outside the nasal cavity; Ecker supposes that he confused them with the nasal cartilages found in this situation, and that Dugès correctly observed them inside the nasal cavity. The next account is found in the early paper of W. K. Parker ('72), (to which reference has already been made).[4] In his paper on the Skull of the Common Snake ('79) he suggests the homology of the septomaxillary of the amphibians and reptiles with the preorbital bone of the ganoid and siluroid fishes: "muco-dermal bones," "specialised in the Amphibia and Reptiles to assist in walling in and flooring of the more perfect nasal labyrinth."

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[1] This paper is no. 148 of the contributions from the Department of Zoölogy of Smith College.

[2] "Des Cornets:—Nous nommons ainsi deux petits os, formés d'une lamelle repliée, courbés en plusieurs sens, situés au côté interne et a l'extrémité antérieure de chaque maxillojugal. Ces osselets sont suspendus dans un cartilage qui n'est q'une dépendance de l'ethmoïde, et cette circonstance est déjà bien favourable à notre détermination. Ajoutez à cela leur forme, leur situation, comparée à celle des cornets des lézards, et l'analogie deviendra évidente; elle ne l'est pas moin si on les compare à ceux de la cécelie que nous avons trouvés attachés au devant de son ethmoïde. Toutefois M. Cuvier les regarde comme des os nasaux rudimentaires et déformés."

It was described by Born ('76) as a lacrimal because of its relation to the nasolacrimal duct, and he homologizes it with the uniform prefrontal of the urodeles, explaining its more complicated form in the anurans as due to the general process of infolding which has affected the structure of the entire nasal region in the group. In his revision of Ecker's and Wiedersheim's "Anatomie des Frosches," Gaupp ('04) remarks that the position of the bone inside the nasal cavity does not make a homology with the lacrimal bone probable. He therefore gives it the new name of 'Intranasale.' In 1905, he again attacks Born, this time on the homology of the bone with the prefrontal of the Urodeles. He points out that the inaccuracy of this view is shown in the fact that some urodeles possess a 'septomaxillary' in the same position as in the anurans besides a typical prefrontal. Ample evidence for this statement has been furnished by Parker in his numerous urodelan studies, and also by later workers. Gaupp concludes by saying that he regards the homology of the septomaxillary in the anurans, urodeles, and sauropsida as settled, and renounces his term 'Intranasale,' in favor of the older designation of Parker.

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[3] See Haslam's translation of Ecker's "Anatomie des Frosches." Oxford, 1889.

[4] "The septo-maxillary is not easy to understand; it appears *above* as a little grain of bone jammed in between the nasal process of the pre-maxillary and the facial plate of the maxillary in front of the outer nostril. *Below*, it is seen as a little curled spicule of bone in the inner nostril. A transversely vertical section seen from behind, shows that it is more than half a tube, lining the front of the nasal passage and sending down a curled process which can be seen from the palate. Another section shows how it rests upon the nasal cartilage, outside, close behind the little valvular flap which partly occludes the external nostril."

Born also homologized the septomaxillary with the lacrimal of the Amniota; this was questioned by P. and F. Sarasin (Gaupp, '05), and by Peter ('98), whose hypothesis that the septomaxillary corresponds to an anterior or 'front' prefrontal has not received much support. The fact that the Sauropsida possess, besides a prefrontal and a lacrimal which is perforated by a nasolacrimal duct, also a septomaxillary in a similar position to that in the Anura is opposed to this view of Born.

The first paper entirely devoted to the consideration of the septomaxillary was published by Hugo Fuchs ('11). His main thesis is the homology of the septomaxillary of the Amphibia and Reptilia, which he treats only in summary manner, with the anomalous nasal floor bone of Dasypus among the mammals. He bases his homology to a certain extent on paleontological evidence. The fact that the bone is missing in the Anomodontia, but occurs in the Therocephalia and probably in all the Cynodontia, strongly supports the probability that the promammals also possessed the bone. The proof of the conjecture that the bone persists from the promammals to the mammals, exists, he believes, in the 'nasal floor bone' of Dasypus first described by Broom who suggested the homology, but only gave it very slight consideration. Fuchs investigated the embryology of the bone in Dasypus and found it similar in many respects to Hatteria and Lacerta, especially to Hatteria in its relations with the nasolacrimal duct.

He refers to Gaupp's theory that the septomaxillary in mammals is fused with the premaxillary; the grounds for the hypothesis being the independent origin of the two parts of the premaxillary, the processus palatinus and the processus extranasalis, the latter being the supposed fate of the septomaxillary. Dasypus, however, has a processus extranasalis praemaxillaris as well as a septomaxillary, near which it arises. One must therefore of necessity assume that the processus extranasalis is of diverse origin in the monotremes and ditremes. Fuchs does not accept this, and in consequence cannot recognize a septomaxillary in the processus extranasalis for the Ditremata.

Regarding the septomaxillary in the urodeles, although in his various papers on the group, Parker figures and describes it with much accuracy as a little 'graniform' structure in relation to the maxilla, in Amblystoma, Desmognathus fusca, Triton cristatus,[5] Salamandra maculosa,[5] Notopthalmus viri descens, Cynops pyrrhogaster and Spelerpes rubra, Wiedersheim ('77), Cope ('89), and other writers in the last quarter of the past century omit the bone in many of their figures and descriptions. Bruner ('01 b), in a description of the general anatomy of the nasal region of the Salamandrina, refers to the bone under the name of 'intranasale' in some detail.[6] This author also describes the nasal muscles and their relation to the bone, but with certain inaccuracies as will be seen later.

Noble ('21) remarks that "Its presence or absence should have as much morphogenetic significance as the presence or absence of the prefrontal. . . . There still remains to be pointed out the wide occurrence of this element in the sala manders and the possible significance of its occasional absence.''

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[5] I have been unable to find any trace of the septomaxillary either in Salamandra maculosa or in Triton helveticus (see description and figures).

[6] "'From the caudal end of the nasal muscle thickening [in Triton alpestris], a slight prominence, representing the turbinal of Wiedersheim and Born, extends to the posterior wall of the nasal cavity. The turbinal is supported by the planum terminale, which is slightly bent into the nasal cavity. In certain species (Plethodon erythronotus, Amblystoma tigrinum, Desmognathus fusca) this turbinal is strongly developed, and its anterior end is supported by a small bone, the intranasale, which lies in the caudal end of the fenestra rostrolateralis. In Plethodon the intranasale has a simple tubular form and its caudal margin lies in close contact with the edge of the planum terminale, excepting where it is excavated to provide a passage for the ductus naso-lacrymalis. In Amblystoma occurs a similar, but much deeper, excavation and the intranasale, which is larger in this species, is thereby divided into two arms, one of which lies above and medial, the other below and lateral, from the ductus nasolacrymalis. The dorsal arm of the lacrymale lies in contact with the cartilago obliqua, the ventral arm rests on the floor of the cartilaginous nasal capsule."

H. H. Wilder ('23) speaks of the septomaxillary, ''found as a cartilage bone in the midst of the very complicated cartilaginous nasal network of frogs and toads.''

Kingsley ('25) refers to the septomaxillaries as 'cranial membrane bones.'

I. W. Wilder ('25) figures the element in Eurycea bislineata as a cartilage bone and describes it as follows:

> The latero-posterior margin of the external nasal aperture of the cartilaginous capsule is strengthened by a small bone, the homology of which is, in my opinion, by no means established but which is commonly called the 'septo-maxillary.' This bone, which in Eurycea, at least, bears a definite relationship to the muscular apparatus for opening and closing the naris, is here of wholly cartilaginous origin although it has been repeatedly referred to by various authors (Parker, 1871; Gaupp, 1905; Noble, 1921) in this or related forms as one of the dermal bones.

The homology of the anterior cranial elements of the vertebrate skull in the various groups still provides material for much controversy among comparative anatomists. We find the literature appertaining to the septomaxillary suggestive rather than conclusive. The following investigation attempts the determination of the nature of this bone, its occurrence and anatomical relations in the Amphibia Urodela.

I wish to thank Mrs. I. W. Wilder for the loan of sections and the provision of certain material and also for her kind interest; Dr. E. R. Dunn for material; and Dr. H. H. Wilder, under whose direction the work has been carried out, for valuable advice and criticism.

## MATERIALS AND METHODS

The method of procedure, in brief, has been the combination of observations of in toto preparations with the study of serial sections. Preparations of the skulls by Noble's modification of the Schultze KOH method (the staining of the bone with alcoholic alizarine before clearing in glycerin) were made in all possible cases, and the septomaxillary thus studied in its relationship to the other bones of the skull as a complemental whole. Some of the specimens used for these in toto preparations were fresh,[7] but many were preserved in formalin,[8] making the process of preparation much longer.

Where the size of the animal has made it practicable (incidentally, this has included all the examples found to possess a septomaxillary), the above method has been followed by the study of serial sections in transverse and occasionally (where material has permitted) in horizontal and parasagittal planes. The fresh specimens were killed in chloretone or chloroform and fixed in Bouin's fluid. For decalcification, a saturated solution of picric acid to which 1 per cent $HNO_3$ had been added, was used in all cases. The sections, which were cut either 10 μ or 15 μ thick, were stained on the slide with Delafield's haematoxylin counterstained with eosin. Bruner's nomenclature has been adopted to a large extent.

In working through the Urodela by these methods, I have used Dunn's classification ('22) and have examined some thirteen examples, representative of the different families, for the presence of the septomaxillary bone.

Mutabilia
  Salamandroidea

| | |
|---|---|
| 1. Amblystomidae, | Dicamptodon, Rhyacotriton, Amblystoma.[9] |
| 2. Salamandridae, | Salamandra,[9] Chioglossa Tylototriton, Pachytriton, Pleurodeles, Triturus,[9] Salamandrina.[9] |
| 3. Plethodontidae, | Desmognathus,[9] Leurognathus, Plethodon,[9] Ensatina, Hemidactylium,[9] Aneides, Batrachoseps, Stereochilus Typhlotriton, Typhlomolge, Gyrinophilus, Pseudotriton, Eurycea,[9] Hydromantes, Oedipus, Oedipina. |

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[7] Desmognathus, Eurycea, Necturus.

[8] Amblystoma, Salamandra, Amphiuma, Hynobius, Cryptobranchus, Siren.

[9] Used as examples in this paper.

| | |
|---|---|
| Amphiumoidea (relationships uncertain, possibly should stand as a family under Salamandroidea) | |
| 4. Amphiumidae, | Amphiuma.[9] |
| Cryptobranchoidea | |
| 5. Hynobiidae, | Hynobius,[9] Pachypalaminus, Onychodactylus, Ranodon, Batrachuperus. |
| 6. Cryptobranchidae, | Megalobatrachus, Cryptobranchus.[9] |
| Proteida (relations uncertain) | |
| 7. Proteidae, | Necturus,[9] Proteus.[9] |
| Meantes (relationships uncertain) | |
| Sirenidae, | Siren,[9] Pseudobranchus. |

## DESCRIPTION

### *Amblystomidae, e.g., Amblystoma maculatum*

In the cleared skull preparation (fig. 2) the septomaxillary appears as an elongated, curved ossification in the nasal capsule, in the angle between the nasal bone and the maxillary. The prefrontal, which touches the apex of this angle, is pierced by a canal which proves to be in line with the canal provided by the septomaxillary. This[10] is the course of the nasolacrimal duct, whose elongated, markedly anterior opening into the nasal sac is completely protected by the 'wrapping round' of the septomaxillary (fig. 3). The actual entrance of the duct into the nasal capsule is through an excavation between the two posterior arms of the septomaxillary and the planum terminale. The general relationships of the nasal muscles are of the type common to the Salamandroidea (fig. 1), though they are comparatively larger in size: the septomaxillary ossification, which serves for their partial origin, is more extensive, probably, in correlation with this fact.

The crescent-shaped musculus constrictor naris, which bounds the posterior border of the external nasal opening and covers in the posterior border of the fenestra rostrolateralis, is inserted medially in the cartilago obliqua and laterally in the subcutaneous connective tissue and in the upper surface of the septomaxillary: it tapers posteriorly, becoming smaller and smaller, connecting the upper, inner edge of the septomaxillary and the cartilago obliqua. The musculus dilatator naris accessorius arises from the outer convex surface of the septomaxillary and extends anteriorly to its insertion in the posterior lateral border of the external nasal opening. While this muscle is external to the nasal capsule, the musculus dilatator naris proper is internal: the latter arises from the planum terminale just behind the level of the anterior end of Jacobson's organ, and also in part from the upper medial edge of the septomaxillary. It passes forward directly below the constrictor naris, and just lateral and ventral to the cartilago obliqua, until it reaches its point of insertion in the medial posterior border of the external nasal opening.

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[10] For development, see Noble ('21).

The nasolacrimal duct, of which the relation to the septomaxillary has already been described,[11] opens into the posterior ventral corner of the fenestra rostrolateralis. The anterior extremity of Jacobson's organ lies behind this opening, and the organ, lying in the fenestra lateralis is protected by the ascending ramus of the maxilla.

The septomaxillary is an ossification of the anterior border of the planum terminale at the posterior border of the fenestra rostrolateralis; the continuity of bone and cartilage is seen in only three or four of the posterior sections (in the transverse plane), probably owing to the fact that in Amblystoma the bone shows a much higher development than in the other forms with a consequent obscuring of its derivation. The process of ossification of the cartilage must therefore be sought in the larval and metamorphic stages.

### *Salamandridae, e.g., Salamandra maculata, Triton helveticus (Triturus), Salamandrina perspicillata*

In his study of the morphology of the skull in the Amphibia urodela, Parker ('82 b) figures Salamandra maculosa (maculata) with septomaxillary between the premaxillary and the maxillary, saying that "at the junction of these bones, and outside the external nostril, there is a small graniform septomaxillary.'' In my study of three examples from Baden, Germany, I have been unable to find a trace of the element either in the cleared in toto preparation of the skull (fig. 4) or from sections (fig. 5). The three nasal muscles are present: the musculus constrictor naris lies round the posterior lateral border of the external naris (as in Amblystoma); it is large and thick, arising from the lateral and dorsal nasal cartilage surrounding the fenestra rostrolateralis, and in part from connective tissue: the musculus dilatator naris accessorius is here very short and does not extend posteriorly as far as the constrictor muscle; it arises from the ventral nasal cartilage surrounding the fenestra rostrolateralis and passes anteriorly to be inserted in the lateral posterior border of the external naris: the musculus dilatator naris, which lies exclusively inside the nasal capsule, arises from the internal surface of the planum terminale and, with the covering of nasal tissue, forms the so-called 'turbinal prominence' of the nasal cavity; it passes anteriorly underneath the constrictor naris to be inserted in the posterior border of the external naris just dorsal and internal to the dilatator naris accessorius. It seems possible that Parker may have mistaken a projection or splinter of the maxillary for a septomaxillary, the older methods of study giving many opportunities for faulty observation.

---

[11] See also Bruner's description, quoted in the introduction.

The nasal region of Triton helveticus shows many similar features to that of Salamandra maculata (compare figs. 5 and 6): the septomaxillary bone is lacking, but in addition the dilatator naris accessorius is almost entirely atrophied.

My material of Salamandrina perspicillata is rather meager: from an alizarin preparation of a dried specimen restored by the KOH method (figs. 7 and 8), I was able to study in toto the details of the skull. A fragmentary series of old sections begins at about the region depicted for Triton and Salamandra, and there is no trace of the dilatator naris accessorius, which, according to Bruner, is absent. The dilatator naris is very small. Thus in the Salamandridae, so far as studied by me, is seen not only an absence of the septomaxillary, but also a range of variation from a form in which the musculus dilatator naris accessorius is comparatively large to a form in which it is wholly lacking.

### *Plethodontidae, e.g., Desmognathus fusca, Eurycea bislineata, Hemidactylium scutatum, Plethodon cinereus*

Of the four examples of this group, Plethodon has been studied the most extensively, transverse, horizontal, and parasagittal sections having been used to check observation of in toto preparations (fig. 11).

The septomaxillary appears as a lateral ossification of the nasal cartilage and bears close relationship to the three nasal muscles. The musculus constrictor naris is crescent-shaped and lies in the posterior portion of the fenestra rostrolateralis, occupying the posterior border of the external nasal opening. According to Bruner ('01 b), it is inserted medially in the margin of the cartilago obliqua; though my preparations show this to be true anteriorly, more posteriorly the majority of the fibers are inserted into the connective tissue of the nasal epithelium or in the underside of the cartilago obliqua. Variations in the skulls of the different species of Plethodon may, however, allow the veracity of both these observations. Laterally, the muscle is inserted in the ventral margin of the nasal cartilage. Posteriorly, it is flanked by the septomaxillary which joins the cartilago obliqua and the ventral nasal cartilage, being an ossification of the posterior border of the fenestra rostrolateralis. The musculus dilatator naris lies inside the nasal capsule, originating in the anterior end of the planum terminale, dorsal to the opening of the nasolacrimal duct into the nasal cavity. It passes forward dorsally, lying underneath the cartilago obliqua; then, interrupting the nasal tissue, dividing the organ of Jacobson from the main nasal sac, it continues beneath the constrictor naris to its insertion in the posterior medial border of the external opening. The musculus dilatator naris accessorius (figs. 9 and 10) arises from the ventrolateral portion of the bridge which separates the fenestra rostrolateralis from the fenestra lateralis, from the septomaxillary (os intranasale of Bruner), an ossification of the anterior border of this bridge, and in a slight measure from the ventral nasal cartilage. This muscle is exclusively external to the nasal capsule; it passes over the anterior border of the septomaxillary, external to the dilatator naris, and over the dorsolateral of Jacobson's organ to be inserted in the posterolateral margin of the nasal opening.

The nasolacrimal duct passes over the prefrontal and, turning back on itself slightly, bends into the nasal capsule, piercing the cartilage at the posterior corner of the fenestra rostrolateralis. The opening is thus bordered slightly anteriorly and medially by the septomaxillary.

The septomaxillaries of Desmognathus fusca (figs. 12 and 13) Eurycea bislineata (figs. 14 and 15), and Hemidactylium (fig. 16) have very similar relations to the nasal muscles, and the description for Plethodon is applicable with very little modification. The size of the bone varies considerably in the family: very large in Hemidactylium,[12] it diminishes somewhat in Plethodon, is smaller in Eurycea, and is minute in Desmognathus, where the dilatator naris accessorius is correspondingly very small.

Bruner ('01 b) classed Spelerpes (Eurycea) apart from Plethodon, saying that in Salamandrina, Spelerpes, and Diemictylus, the dilatator naris accessorius is absent. My investigations have shown this to be untrue for Eurycea (Spelerpes) bislineata, there being definitely such a muscle with function and relations comparable to the condition found in Plethodon, though the septomaxillary in this case is rather smaller and the muscle insertion less extensive.

### *Hynobiidae, e.g., Hynobius leechii*

The condition of the septomaxillary bone in Hynobius (figs. 17 and 18) shows considerable resemblance to that in Amblystoma; it lies in the posterolateral corner of the fenestra rostrolateralis and shows continuity with the ventral nasal

[12] See Noble ('21).

cartilage. In all probability, it arises as an ossification of the posterior wall of the fenestra rostrolateralis, as in the Plethodontids. It takes the form of a short horizontal tube, the sides being longer than the dorsal and ventral surfaces, both anteriorly and posteriorly, owing to the fact that the nasolacrimal duct enters the tube posterodorsally and leaves it to enter the nasal sac anteroventrally.

The m. constrictor naris is a large muscle curving round the posterior border of the external narial opening; the transversely running posterior fibers insert laterally in the medial border of the septomaxillary. The course of the muscle is ventrolateral to the cartilago obliqua, and it diminishes in size posteriorly in the connective tissue beneath the planum terminale. The m. dilatator naris is noticeably small and short, apparently arising in the inner fibers of the constrictor naris and passing forward to be inserted in the posterior border of the external naris. The smallness of this muscle is, in all probability, correlated with the large size of the dilatator naris accessorius, which arises more posteriorly from the cartilaginous bridge joining the cartilago obliqua and the ventral nasal cartilage, and in part from the outer border of the septomaxillary; it is inserted in the posterolateral margin of the external naris.

*Amphiumidae, e.g., Amphiuma tridactylum. Cryptobranchidae, e.g., Cryptobranchus allegheniensis. Proteidae, e.g., Proteus anguinus; Necturus maculosus. Sirenidae, e.g., Siren lacertina*

As regards the other four families of the Urodela, the Amphiumidae, the Cryptobranchidae, the Proteidae, and the Sirenidae, I have examined the skulls of representative examples as listed above by the alizarine and KOH method and find the septomaxillary wanting in all cases. I. W. Wilder ('09), in summarizing her paper on "The lateral nasal glands of Amphiuma," says: "Among the lower urodeles Proteus and Amphiuma alone possess the homologues of external nasal glands, while the occurrence of nasal constrictor and dilatator muscles is limited to Cryptobranchus and Amphiuma.''

She also gives a very comprehensive description of these muscles which I have reviewed from sections through the nasal region of these forms. In addition, I have been able to examine serial sections through this region in the other examples cited.

Only in Cryptobranchus and Amphiuma, as aforementioned, is the musculature organized to any appreciable extent, and even here I was unable to perceive any development which could be regarded as the more external, accessory, dilatator muscle.

### *The nature of the septomaxillary bone*

Hitherto, the septomaxillary has been classed, by most authors who have described it, as a membrane bone, along with the other membrane bones of the skull. Parker, in his various papers, places it in his description of the maxillaries, prefrontals, nasals, parietals, etc., obviously assuming it to be of the same origin; Gaupp ('05) describes it under the heading 'Deck-knochen.' On the other hand, H. H. Wilder ('23) and I. W. Wilder ('25) suggested its cartilaginous origin.

From the close study of serial sections through the nasal region of various forms, the intimate connection between this bone and the nasal cartilage makes this latter theory more probable. The continuity of the bone with the cartilage in the case of the septomaxillary shows an almost identical relationship with that of the developing orbitosphenoid, a recognized cartilage bone, leading one to the conclusion that the septomaxillary is a hitherto unrecognized cartilage bone or ossification of the cartilaginous primordial skull. The smallness of the bone in most forms and the lateness of its development make the observation of stages in the process of the ossification rather difficult; but the fact that none of the other membrane bones are ever seen in contact or in continuity with cartilage and are always separated from it by flattened connective-tissue cells makes good supporting evidence for the theory. Figures 19 and 20 show the comparative ossification of the orbitosphenoid and septomaxillary of Plethodon and Eurycea, as seen in transverse section. The condition is likewise apparent in Hemidactylium, Desmognathus, and others. The ossification of the prootic and the opisthotic in the cartilaginous otic capsule shows exactly similar continuity between the bone and cartilage.

This consideration of the nature of the bone brings up a very deep problem of morphology, namely, the origin of the bony skeleton. It is now generally accepted that the more superficial bones of the vertebrate skull have arisen in the course of evolution from the basal plates connected with placoid scales. This spreading inward of bone-forming activity in the connective tissue seems to be possible for relatively great distances. Thus a possibility presents itself that the deeper parts of the bony skeleton may also have arisen in evolution by the spreading inward of bone-forming activity from the skin. At the present moment, therefore, it is hardly wise to draw any line between cartilage or 'replacement' bones and membrane or 'skin' bones. I wish, however, to point out that direct ossification of the cartilage in the nasal region gives rise to the septomaxillaries in certain Urodela, and makes them, in the terminology of the old classification 'replacement' bones. It is possible that in the higher forms there may be both an ossification of the cartilage and a laying down of bony tissue of dermal origin, thus producing a combination of 'replacement' and 'skin' bone. The line dividing the two is thus very arbitrary.

## SUMMARY

While the septomaxillary appears to be entirely lacking in the Amphiumoidea, the Proteida, and the Meantes, it is present in the Salamandroidea and in the Cryptobranchoidea. The former of these two groups possesses the bone in two of its three families, namely, the Amblystomidae and the Plethodontidae, but not in the Salamandridae; the latter in the Hynobidae, but not in the Cryptobranchidae.

From the study of the bone in its greater and lesser development, and observations of the corresponding size of the nasal muscles, it seems that the function of the bone is primarily for the origin of the musculus dilatator naris accessorius, since this is reduced in such forms as Salamandrina, Triton, and Salamandra, where the bone is absent. This condition has been explained as "due to a crowding out by the ascending ramus of the maxilla or the nasal which have apparently shifted forward" (Noble, '21); but in the abovementioned forms, these elements show no tendency to take over the function of the lost bone, the accessory dilatator muscle, if present, taking its origin from the ventral nasal cartilage. In forms where it has become more highly developed in correlation with the increased size of the muscle, its proximity to the nasolacrimal duct has been the cause of its 'wrapping round' (a term commonly used in the older descriptions), thus apparently serving secondarily as a means of protection for this structure.

The occurrence of the bone in certain groups of Urodela and not in others falls into line with the following observation and classification by Higgins ('19) on the basis of the nasal capsule:

> The nasal capsules of several families of Urodeles afford a basis for the division of this order of Amphibia into four groups, founded upon similarity of larval structures and the method of chondrification. . . . Of these groups, Spelerpes, Plethodon and Amblystoma form one; Salamandra, Triton and Diemictylus another; Amphiuma, although remote from Cryptobranchus in the later stages, is included with it in the third; while Necturus remains alone in the fourth group.
>
> To recognize in the nasal capsules of the Urodela a complete phylogenetic development or gradual transition from one animal to another is impossible, for many gaps exist concerning which evidences of structural relationships are wanting. On the other hand many resemblances in the development of certain structures in the nasal capsule may throw some light upon the inter-relationships of this Amphibian order.
>
> Of the Urodeles included in this study, it would seem as if the capsule of the American species of Cryptobranchus possesses char-

acters most ancestral and which show relationships to both Urodela and Anura. Regarding Cryptobranchus, then, as more primitive, Spelerpes, Plethodon and Amblystoma appear in an ascending series from the more primitive condition; while Salamandra, Triton and Diemictylus are separated from them, but possibly related to them through some form like Spelerpes, Necturus may be regarded as a neotenic condition of Spelerpes, while Amphiuma is possibly reduced from the more primitive Cryptobranchus.

Without entering here into the discussion of certain implied interrelationships of the various Urodela in their evolution, concerning which, were other data considered, there would undoubtedly be considerable difference of opinion, it is significant to note that of the four groups of the urodeles established by Higgins, upon the basis of the cartilaginous nasal capsule, only one, according to my own observations, possesses the septomaxillary. The "ascending series from the primitive condition" in Spelerpes (Eurycea), Plethodon, and Amblystoma is seen in the development of the bone in the respective examples.

In conclusion, the septomaxillary of Urodela is found to be an ossification of the cartilage in the posterior wall of the fenestra rostrolateralis which provides primarily for the partial origin of the musculus dilatator naris accessorius, and, secondarily, in the higher development, wraps round the nasolacrimal duct.

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## ABBREVIATIONS

*a.n.e.*, apertura naris externa
*c.m.*, cavum medium
*c.mx.*, cavum maxillare
*c.o.*, cartilago obliqua
*c.s.*, cavum superius
*d.nl.*, ductus nasolacrymalis
*ex.*, exoccipital
*f.d.*, fenestra dorsalis
*f.l.*, fenestra lateralis
*f.rl.*, fenestra rostrolateralis
*f.*, frontal
*gl.i.*, glandula intermaxillaris
*gl.n.e.*, glandula nasalis externa
*j.o.*, Jacobson's organ
*lac.*, lachrymal
*l.i.*, lamina inferior
*l.s.*, lamina superior
*m.c.n.*, musculus constrictor naris
*m.d.n.*, musculus dilatator naris
*m.d.n.a.*, musculus dilatator naris accessorius
*mx.*, maxilla
*n.*, nasal
*os.*, orbitosphenoid
*p.*, parietal
*pet.*, petrosum
*p.f.*, prefrontal
*p.m.n.o.*, posterior margin of nasal opening
*pmx.*, premaxilla
*p.t.*, planum terminale
*pt.*, pterygoid
*qu.*, quadrate
*q.j.*, quadratojugal
*se.*, sphenethmoid
*smx.*, septomaxillary
*sq.*, squamosal
*tect.nas.*, tectum nasale
*t.*, turbinale
*v.*, vomer

## PLATE 1

### EXPLANATION OF FIGURES

1 Diagram to illustrate the arrangement of the nasal muscles of the Salamandroidea and their relations to the cartilaginous nasal capsule. Modified after Bruner ('01).

2 Dorsal view of the skull of Amblystoma maculatum. × 6.

3 From Amblystoma maculatum. × 12. Transverse section through the nasal region to show the arrangement of the muscles and the septomaxillary.

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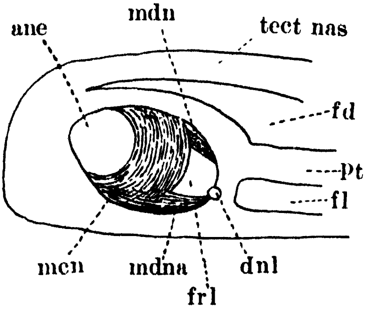
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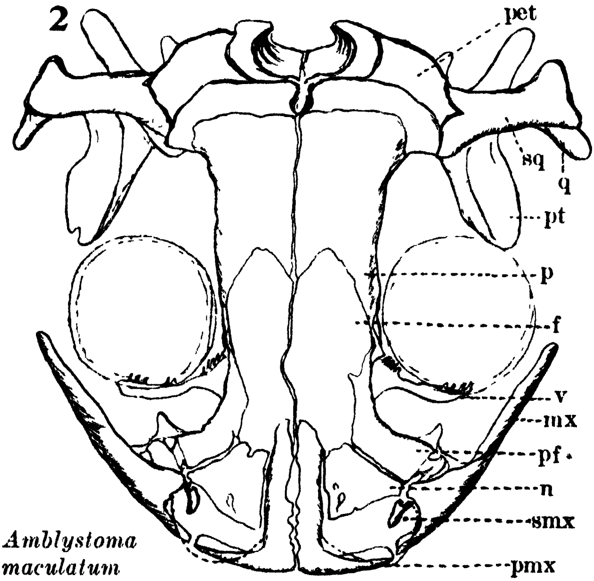
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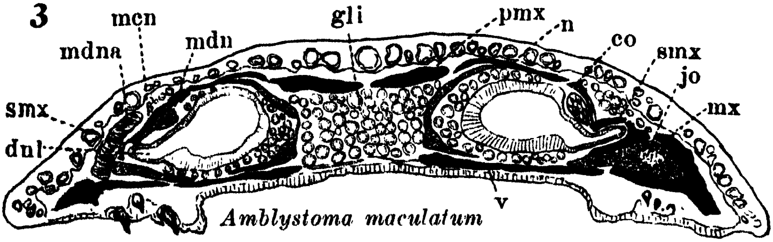
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## PLATE 2

### EXPLANATION OF FIGURES

4 Dorsal view of the skull of Salamandra maculata. × 6.6.

5 From Salamandra maculata. × 16. Transverse section through the nasal region to show the arrangement of the nasal muscles.

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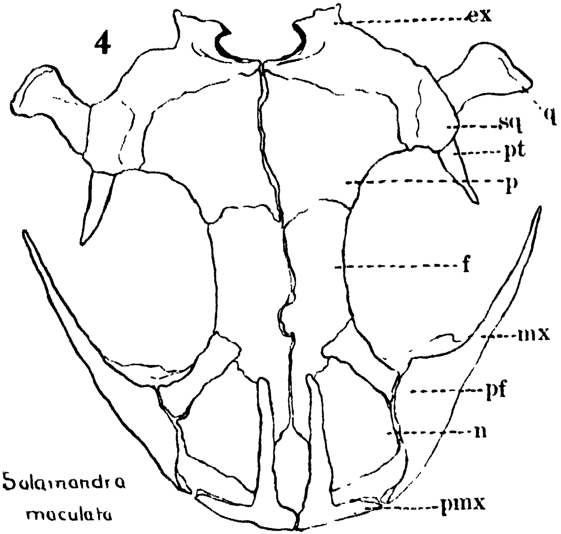
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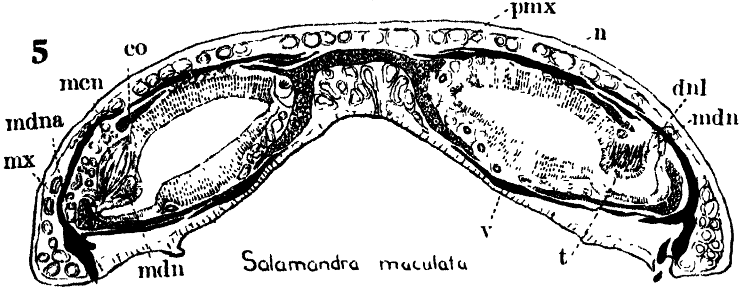
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# PLATE 3

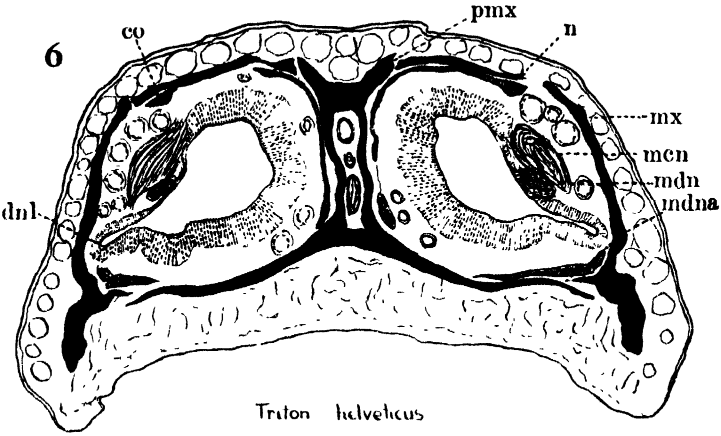
## EXPLANATION OF FIGURES

6 From Triton helveticus. × 32. Transverse section through the nasal region, to show the arrangement of the nasal muscles: the musculus dilatator naris accessorius is here very small.

7 Dorsal view of the skull of Salamandrina perspicillata. × 7.2.

8 Anterior view of the same. × 7.2.

6



Triton helveticus

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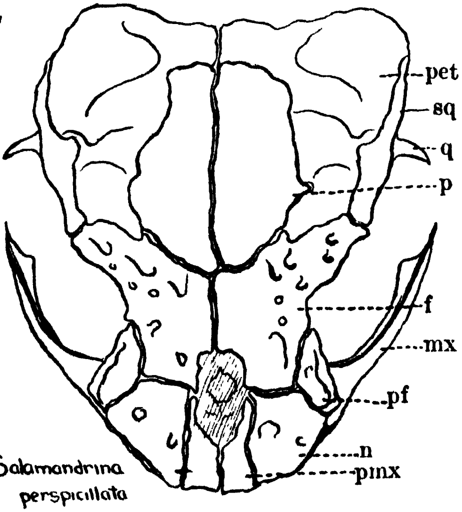

Salamandrina perspicillata

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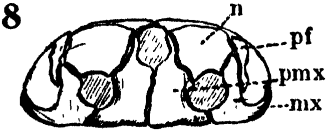


Salamandrina perspicillata

## PLATE 4

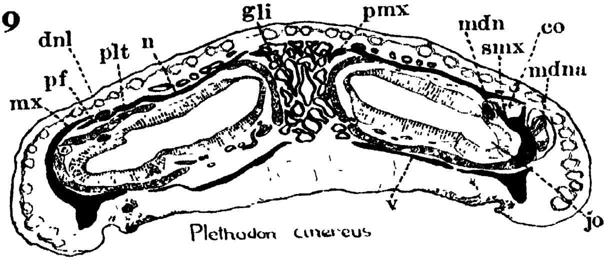
### EXPLANATION OF FIGURES

9 From Plethodon cinereus. × 26.4. Transverse section through the nasal region, to show the dilatator muscles and the septomaxillary.

10 From Plethodon cinereus. × 46. Horizontal section through the lower nasal region, showing the septomaxillary and the musculus dilatator naris accessorius.

11 Dorsal view of the skull of Plethodon cinereus. × 9.6.

9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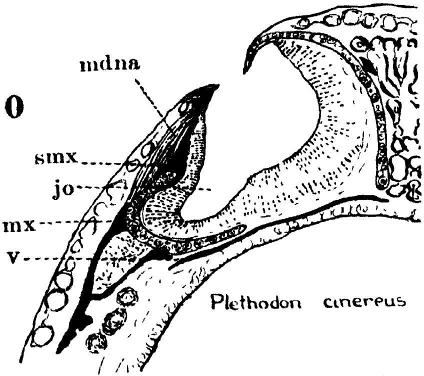
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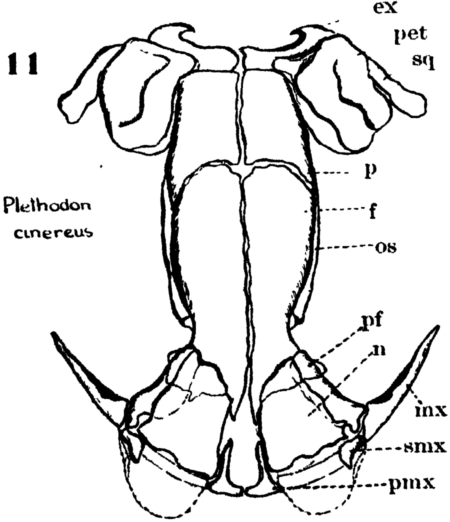
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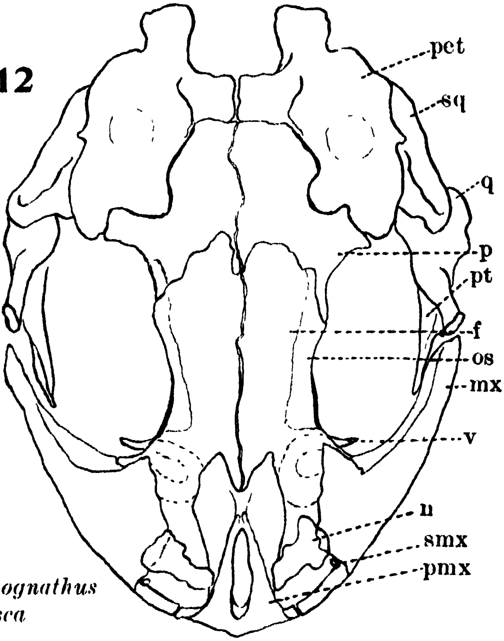
# PLATE 5

## EXPLANATION OF FIGURES

12 Dorsal view of the skull of Desmognathus fusca. × 7.

13 From Desmognathus fusca. × 22. Horizontal section through the nasal region, showing the relationship between the muscles and the septomaxillary.

12



*Desmognathus fusca*

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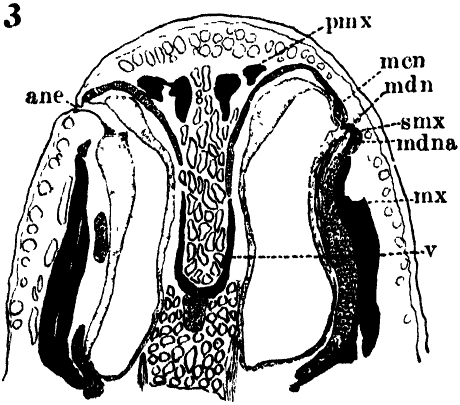


*Desmognathus fusca*

## PLATE 6

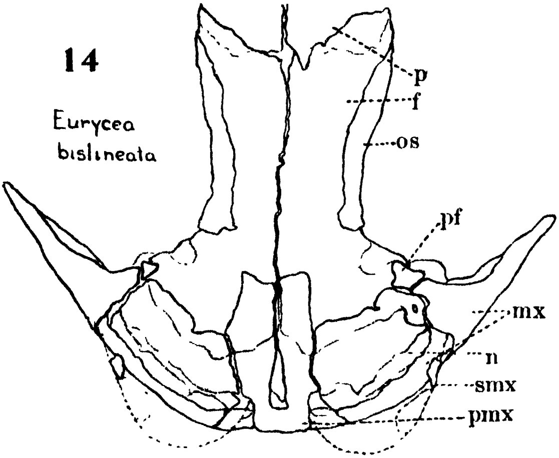
### EXPLANATION OF FIGURES

14 Dorsal view of the anterior cranial elements of the skull of Eurycea bislineata. × 8.4.

15 From Eurycea bislineata, young adult. × 36. Transverse section through the nasal region, to show the septomaxillary and the arrangement of the muscles.

16 From Hemidactylium scutatum. × 32. Transverse section through the nasal region, to show the septomaxillary and the dilatator muscles.

14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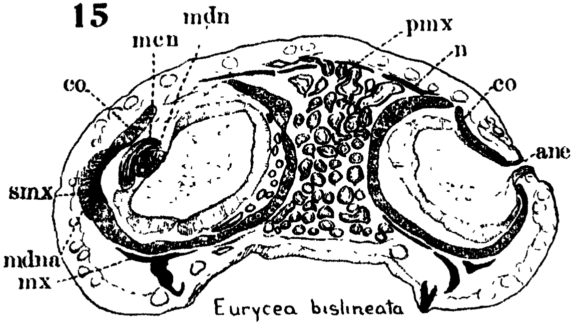
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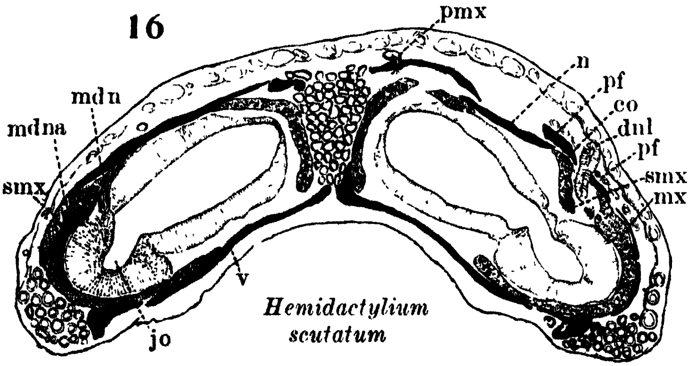
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Hemidactylium scutatum

## PLATE 7

### EXPLANATION OF FIGURES

17 Dorsal view of the skull of Hynobius leechii. × 7.8.

18 From Hynobius leechii. × 22. Transverse section of the nasal region, showing the relationships between the septomaxillary, the muscles, and the nasolacrimal duct.

19 From Plethodon cinereus. × 51. The process of ossification in young adult specimens Transverse sections, showing the continuity of the cartilage and bone in, (A and B) septomaxillary, (C and D) orbitosphenoid.

20 From Eurycea bislineata. × 51. The process of ossification in, I) The orbitosphenoid of an advanced metamorphic specimen; II) The septomaxillary of an adult.

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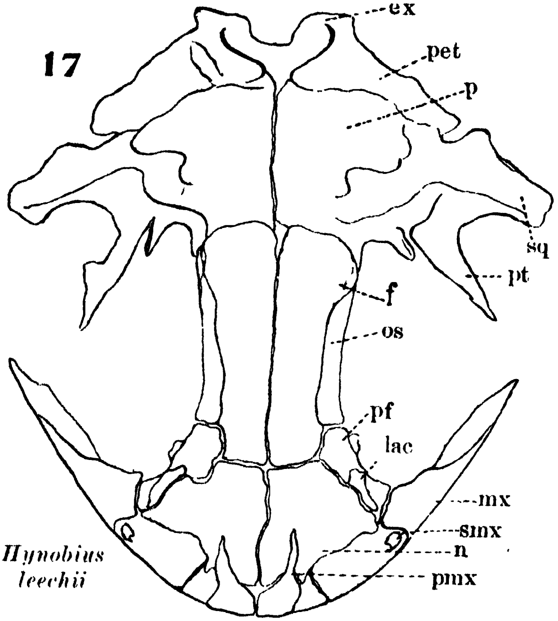
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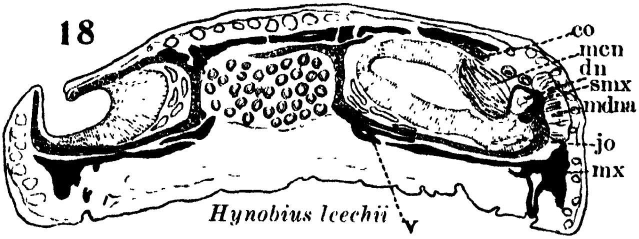
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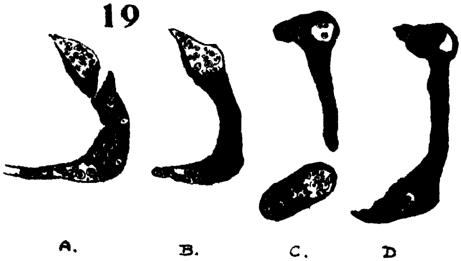
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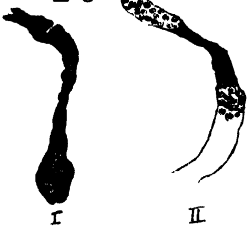
I
II

# THE ANATOMY AND LIFE-HISTORY OF A FRESH-WATER MOLLUSK OF THE GENUS SPHAERIUM[1]

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TWENTY FIGURES

AUTHOR'S ABSTRACT

A detailed study has been made of the anatomy of one of the fingernail shells, and preliminary observations on the life-history have been carried out. In its general organization Sphaerium notatum is very similar to the larger fresh-water lamellibranchs. A gastric shield, crystalline style, and style sac, very similar to those found in the stomach and intestine of Lampsilis, are present. A pair of slender muscles extending from the dorsal side of the body into the gills, and evidently not previously described, have been found. The nervous system consists of the typical three pairs of lamellibranchiate ganglia, with their connectives, accessory ganglia, and nerve fibers. Particular study was given to the statocysts and osphradia, and attention is called to the fact that the function commonly ascribed to the osphradia is incompatible with their position in the roof of the cloacal chamber.

S. notatum, like all the Sphaeriidae, is hermaphroditic and viviparous. The gonads are paired racemose glands lying behind and below the stomach. The sperm-producing follicles form the anterior portion of each gonad and are somewhat smaller and more numerous than the ova-producing follicles which form the posterior portion. The young pass through the early stages of development in brood pouches in the gills and are expelled as relatively enormous individuals.

Preliminary observations on the life-history indicate that reproduction reaches its height in the summer and that fertilization probably takes place during the late summer and fall.

## CONTENTS

Introduction .... 474
1. Historical .... 474
2. Materials and methods .... 475
3. Acknowledgments .... 476
Systematic position and habits .... 476
Morphology .... 477
1. The shell .... 477
2. The animal .... 477
*a*. The mantle .... 478
*b*. The gills .... 479
*c*. The visceral mass .... 480
*d*. The foot .... 481
a. The mucous glands .... 481
b. The byssal gland .... 482
3. The muscular system .... 482
4. The digestive system .... 484
*a*. The alimentary canal .... 484
*b*. The digestive diverticula .... 485

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[1] Contributions from the Zoölogical Laboratory of the University of Illinois, no. 317.

- *c.* The crystalline style and style sac ........ 486
- *d.* The gastric shield ........ 487
- *e.* The labial palpi ........ 488

5. The nervous system ........ 488
   - *a.* The ganglia and commissures ........ 488
   - *b.* The organs of special sense ........ 490
     - a. The statocysts and statoliths ........ 490
     - b. The osphradia ........ 492
6. The circulatory system ........ 492
7. The excretory system ........ 494
8. The reproductive system ........ 495

The life-history ........ 496

Summary ........ 497

Bibliography ........ 498

## INTRODUCTION

### *1. Historical*

While much has been published on the morphology of the larger fresh-water Acephala, there is but little available on the anatomy of the Sphaeriidae, or finger-nail shells. In 1896, Gilman A. Drew published a short description of the anatomy of Sphaerium sulcatum, and, in 1917, Ralph J. Gilmore contributed a paper entitled: "Notes on reproduction and growth in certain viviparous mussels of the family Sphaeriidae." While Drew gave a very good general description, he failed to describe several important structures. Gilmore confined his attention to an investigation of the gills and the reproductive organs. These are the only papers in English on the anatomy of this family of mollusks.

Even less is available on the life-histories of these forms. Little is known beyond the fact that they are viviparous and that the young pass through the early developmental stages in brood pouches within the gills of the parent. Thiel ('27: 40–47, and '27: 48–90) has recently published two papers on the growth, reproduction, and life-history of Sphaerium corneum, directing attention chiefly to seasonal growth.

It was this lack of information concerning both the anatomy of these forms and their life-history that led Dr. H. J. Van Cleave, of the University of Illinois, to suggest this study.

## 2. *Materials and methods*

*Material.* The material for this study was collected in a drainage ditch at Urbana, Illinois, at various times during the fall of 1926 and the spring and summer of 1927. In the course of the study several hundred adult specimens and many more smaller ones were under observation. Collections of this material submitted to Dr. V. Sterki were identified by him as Sphaerium notatum Sterki (Sterki, '27). While the results presented in this paper are confined to this one species, comparative studies have been made upon an additional species of Sphaerium and also upon an unidentified species of the genus Pisidium. In general morphology, these forms agree so closely with the conditions found in S. notatum that most of the descriptive matter here presented, other than specific measurements, has value as a guide to the study of the Sphaeriidae.

*Methods.* Numerous gross dissections were made under a binocular and many observations on structure and function were made upon young and transparent specimens under the compound microscope. In order to study the anatomy in finer detail, the animals were killed and sectioned whole or in part. It has been found that the best fixation and relaxation were secured by preventing the closing of the shell and plunging the animal into slightly warmed Bouin's fixative. Cold Bouin's, Zenker's, and corrosive sublimate all gave good fixation, but the above method was found to prevent the extreme contraction of the muscles found when a cold fixative was used. The acid in the fixative is sufficient to remove the calcium salts of the shell if it is left on overnight, but I have found it possible to dissect away the shell after the animal has been in the fixative for a short time, without injury to any parts, and at the same time avoiding the gas bubbles which form if the acid is allowed to act. The animals were in the most instances cleared in benzol, embedded in paraffin, and sectioned entire. Sections were cut from 8 μ to 20 μ thick. Iron hematoxylin, counterstained with acid fuchsin, or Delafield's hematoxylin and eosin gave very good differentiation.

Observations on reproduction were carried on by placing small groups of adults in running water in shallow pans containing a layer of screened sand. These aquaria were examined from time to time for the presence of young sphaeriids, which were removed when found.

### 3. *Acknowledgments*

This study has been carried on in the Zoology Laboratory of the University of Illinois, under the direction of Dr. H. J. Van Cleave, to whom I wish to express my appreciation of the valuable assistance he has given me. I also wish to thank Dr. V. Sterki for the identification of my material, and F. C. Baker for additional material from this group of mollusks.

## SYSTEMATIC POSITION AND HABITS

The finger-nail shells belong to that class of mollusks variously known as the Acephala, Lamellibranchia, and Pelecypoda. The family Sphaeriidae includes the three North American genera Sphaerium, Musculium, and Pisidium, and the tropical genus Eupera. Baker ('27: 220) has recently divided the family into two subfamilies, based on the character of the siphons. In the subfamily Sphaeriinae, which includes Sphaerium, Musculium, and Eupera, the anal and branchial siphons are distinct, united only at the base. In the subfamily Pisidinae, which includes the genus Pisidium, the anal siphon only is developed, the branchial siphon being represented by a cleft in the mantle.

The animal is usually found almost completely buried in the mud on the bottom or along the banks of small streams; it is rarely found in sand. Locomotion is accomplished by a series of erratic jerks. The foot is greatly extended and anchored by means of an expansion of the anterior tip and the ventral side. Adhesion is facilitated by a mucous secretion from the mucous glands of the foot. The shell is then drawn forward through the mud by a violent contraction of the foot retractor muscles. The mucous secretion is an important means of anchoring the foot; young specimens readily climb vertical glass surfaces by its aid alone.

S. notatum, as is the rule in the Sphaeriidae, is viviparous. From three to five relatively gigantic young, as well as several smaller embryos, are usually found in each brood pouch in adult animals.

## MORPHOLOGY

### *1. The shell*

The shell of this species is dark brown in color, lighter at the edges, rather solid, inflated, and covered with a thin epidermis. The lines of growth are well marked. The umbones are smooth, depressed and placed slightly in front of the center of the shell. The hinge line is nearly straight; the ligament is dark horn. The cardinal teeth (fig. 1) are very small. There is a single, raised, bent tooth in the right, and two small, elevated teeth in the left valve. The lateral teeth are thin raised lamellae, 2–2 in the right and 1–1 in the left valve, placed at less than right angles with the cardinal teeth. The scars of the adductor muscles show plainly on the nacre, and are joined dorsally by the scars of the retractor pedis muscles. The pallial line is indistinct. Adult specimens measure 14 × 9 mm., with a transverse diameter of 7 mm.

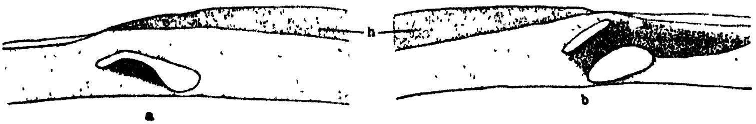

Fig. 1 The cardinal teeth in S. notatum. *a*, in the right valve; *b*, in the left valve; *h*, the hinge line.

### *2. The animal*

The general plan of organization of the body is essentially the same as that characteristic of the whole group of fresh-water Acephala. The tissues of the living animal are creamy white, with the exception of the digestive diverticula, which are light brown. The bilobed mantle fits closely against the valves of the shell. The dorsal part of the mantle cavity is occupied by the gills and the visceral mass, ventral to which extends the protrusible foot.

*a. The mantle.* The mantle (fig. 7, *mtl*) consists of two thin sheets of connective tissue which line the inner surfaces of the two valves. Each sheet, or lobe, is made up of loose connective tissue, covered by an inner and an outer layer of epithelium. The outer layer, lying against the shell, is made up of thin, flat cells, and is continuous over the back with its fellow from the opposite side. The inner layer of epithelium is made up of low cuboidal cells, and is continuous with the epithelium of the outer lamella of the outer gill posteriorly and with that of the outer lamella of the inner gill anteriorly. A short distance from the ventral edge of the mantle this epithelial layer is thrown up into a conspicuous ridge (fig. 9, *c r*) which extends from the anterior adductor muscle to the branchial siphon. Just dorsal to this ridge is a shallow groove (fig. 9, *cgr*), under which the layer of connective tissue is much thinner. The epithelium in the groove and over the ridge is composed of tall, ciliated columnar cells. Immediately ventral to this ridge the epithelium is made up of low cuboidal cells, but nearer the ventral edge of the mantle the cells again become columnar. The cilia are confined to the ridge and the groove above it. Posteriorly, these ridges from each lobe fuse to form the siphons. The connective tissue under the ridges is well supplied with both longitudinal and oblique muscles.

These ridges, with their muscles, serve to close the ventral opening of the mantle cavity when the shell is open, thus forcing the water to enter through the branchial siphon. When the foot is protruded, the ridges are closely applied to its sides. The ciliated grooves evidently serve as channels to carry water forward to the mouth.

Posteriorly, the lobes of the mantle are fused, by means of the ridges described above, to form two siphons. As previously mentioned, the siphons furnish characters of subfamily significance in the Sphaeriidae (Baker, '27 : 220). The ventral or branchial siphon is wider and somewhat shorter than the dorsal, anal siphon. Both siphons are well supplied with muscles and are capable of considerable protrusion; the size and shape of the openings may be readily changed. Near the base of the siphons the mantle muscles are enlarged (fig. 2), and serve to withdraw the extended siphons. The inhalent siphon usually points directly posterior or a little ventrally, while the exhalent siphon points posteriorly and a little dorsally.

At the ventral edge of the mantle there is a deep cleft in which the epidermis is secreted by the epidermal gland. This gland (fig. 9, *ep gl*) is a band of cells about 0.3 mm. wide lying along the outer side of the cleft and extending the length of the edge of the mantle. It is made up of tall columnar cells having large elliptical nuclei near their bases. The outer ends of the cells contain many fine granules.

*b. The gills.* In addition to the function of respiration, the gills carry the developing young in modified structures known as brood pouches. There are four gills, arranged as an inner and an outer pair, suspended from the sides of the body into the mantle cavity. The inner pair is the larger and extends from the anterior adductor muscle to the posterior adductor muscle. In fully grown specimens the inner gills are about 7 mm. long and 3 mm. wide in the widest part. The outer gills are somewhat smaller and extend only three-fourths as far forward. Their anterior and upper limit is near the posterior end of the stomach, from where they extend posteriorly and ventrally to join the inner gills at the posterior adductor muscle.

Each gill is made up of two closely applied plates, an inner and an outer lamella (fig. 19, *i l* and *o l*). The inner lamellae of the inner gills are attached to the body along the extent of the foot. Behind the foot they are attached to each other, thus separating the mantle cavity from the cloacal chamber. The outer lamellae are attached to the mantle anteriorly and to the inner lamellae of the outer gills posteriorly. The outer lamellae of the outer gills are attached to the mantle.

Each lamella is made up of a series of hollow filaments, each filament being bent upon itself to form a slender U, and therefore continuous with its fellow in the opposite lamella. Opposite filaments are fused together by their adjacent sides throughout their length, except in the brood pouches. The water tubes between adjacent pairs of filaments are thus definitely limited. The adjacent filaments in each lamella are regularly united at intervals of 0.25 mm. by interfilamentary junctions. The spaces between these junctions are the inhalent ostia, through which water enters the water tubes.

Each filament (fig. 19, *fil*) is a slender tube covered by a single layer of epithelium. The inner part of the tube is made up of a thin layer of cells, which form a very thin membrane. The outer part is made up of a single layer of very large cells supported by a slender chitinous rod. These outer cells bear numerous cilia. The outer face bears rather short cilia, while the corner cells (fig. 19, *ccc*) bear tufts of long cilia. There are two tufts of cilia on each corner; one on the corner itself, and another just mesial to it. Figure 19 shows a frontal section of the inner gill, showing these tufts of cilia, the interlamellar and interfilamentary junctions, etc.

As previously noted by Gilmore ('17: 22–23), the upper parts of the lamellae of the inner gills are modified to serve as brood pouches (fig. 7, *br po*) in which the embryos develop to a relatively advanced stage. In most individuals the inner gill only serves as a brood pouch, but the outer gill may also be used (fig. 8).

*c. The visceral mass.* The visceral mass is the softer part of the body, lying in the dorsal part of the mantle cavity. It is composed of much loose connective tissue, which surrounds and supports the organs of digestion, circulation, excretion, reproduction, and most of the nervous system. It is covered by a single layer of cuboidal epithelium continuous with that of the gills and mantle.

*d. The foot.* The locomotor organ is the protrusible foot, which is a muscular projection from the ventral side of the visceral mass. It is pointed anteriorly and somewhat compressed laterally. It is capable of great protrusion, often being extended forward a distance equal to the length of the shell. The foot is made up of two groups of interlacing muscle fibers, separated by a narrow vertical cleft in the middle of the foot (fig. 7, *cl*). Each group of fibers is derived from the retractor pedis muscles of its side and is joined to the other by means of many fine strands across the cleft.

The foot is covered by a single layer of tall columnar epithelial cells. These cells have large, deeply staining nuclei near their bases and many minute granules near their outer ends. They do not lie upon a basement membrane; instead, there is a thin layer of muscle fibers, passing in all directions, lying against their inner ends. Over much of the foot these cells bear many fine, short cilia. The cilia cover the ventral surface of the foot and much of the sides. The direction of their action is always upward toward the base of the foot, for when a crawling animal brings its foot into contact with light debris, the debris is always carried upward and posteriorly.

The portions of the foot bearing the cilia are covered by a very fine membrane which is closely applied to the epithelial cells. It is 0.001 mm. thick, and seems to be a protective covering secreted by the epithelial cells described above. It has the appearance of a fine cuticula, but preliminary experiments with artificial gastric juice indicate that it is not a true cuticula.

*a.* The mucous glands. Numerous writers have mentioned the fact that some sphaeriids may be found crawling about on vegetation, but the glands which must produce the necessary mucus seem to be undescribed. Mucus has always been in evidence when even small animals were in watch-glasses for observation.

In the ventral part and sides of the foot next to the external epithelium, the interstices between the muscle fibers are filled with many large mucus-secreting cells (figs. 11 and 17, *mu*). The layer of cells is thickest on the ventral side at the anterior end of the foot, and gradually becomes thinner posteriorly and upward along the sides. These cells are large spherical or ovoid cells having large central vesicular nuclei, and a finely granular cytoplasm.

*b*. The byssal gland. In most of the Acephala the byssal gland is functional, at least in the younger stages, forming a secretion by which the animal is attached. In the Sphaeriidae the gland is vestigial. There is a single spherical vesicle (fig. 17, *by gl*) in the foot a short distance posterior to the pedal ganglia. It is relatively much larger in the embryo, and is supplied with a duct that extends toward the posterior, ventral edge of the foot, giving the gland a pear-shaped appearance. This duct is already occluded in embryos 1 mm. or less in length.

### 3. *The muscular system*

The muscles may be conveniently grouped as adductors, retractors, foot, mantle, and siphonal muscles.

The two adductor muscles, one anterior and the other posterior, extend transversely through the body, pierce the lobes of the mantle, and are inserted into the shell. When these muscles are relaxed the hinge ligament causes the valves to gape open. The anterior adductor muscle (fig. 2) is a thick band, elongate ovoid in cross-section, lying in the dorsal part of the body, immediately anterior to the mouth. It is composed of numerous bundles of fibers which break into fine strands at the insertion into the shell. The posterior adductor is ventral and lies immediately anterior to the rectum (fig. 2). It is likewise composed of transverse bundles of fibers, but is nearly round in cross-section.

All the remaining muscles are paired, there being one member in each side of the body. The anterior retractor pedis muscles (fig. 2) arise just dorsal to the ends of the anterior adductor. The fibers converge below the esophagus, then separate and pass posteriorly and ventrally into the foot, through which they ramify. The posterior retractor pedis muscles (fig. 2) arise dorsal to the ends of the posterior adductor. They converge in front of the adductor, diverge, and pass anteriorly and ventrally into the foot. There they spread out in the same manner as the fibers of the anterior retractors.

The muscles of the foot are separated into right and left halves by a median cleft (fig. 7, *cl*), as previously stated. The lateral components are united at the ventral edge of the foot by an intimate interlacing of their fibers and also across the cleft by many fine strands. The fibers of the foot pass in all directions, but may be grouped as horizontal, transverse, and vertical fibers.

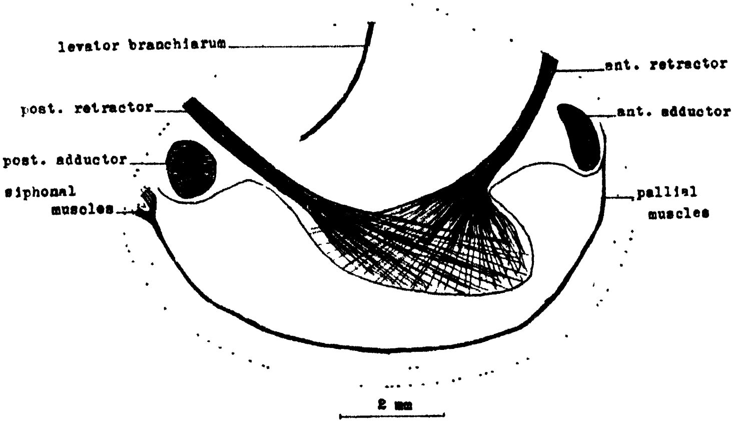

Fig. 2 Lateral view of the muscular system from the right side.

Arising at the dorsal body wall immediately posterior to the stomach are a slender pair of muscles which may be called levator branchiarum muscles (fig. 8, *lev branch*). They are attached to the shell near the umbones. After passing laterally, ventrally, and posteriorly in the septum between the auricles and the epibranchial chambers of the outer gills, they are inserted along the junction of the inner and outer gills.

These muscles have not been previously described. Pelseneer ('06: 218) states that the foot is supplied with a pair of median elevator muscles, but these are evidently not the same, for the muscles in S. notatum pass into the junction of the inner and outer gills in all cases and end there. The levator branchiarum muscles evidently serve to support the gills and to draw them upward.

As previously noted, the ventral edge of the mantle is provided with muscles (figs. 2 and 3). There is a slender band of longitudinal muscles in the connective tissue just above the ciliated mantle ridge (fig. 9, *ll pl m*). There is also a set of muscles (fig. 9, *ob pl m*) which pass obliquely through the mantle to be inserted into the shell along the pallial line.

### *4. The digestive system*

*a. The alimentary canal.* The digestive system consists of the alimentary canal and its derivatives. The labial palpi will be considered here because they are important accessory structures having to do with the selection of food material. The mouth (fig. 7, *mth*) is ventral to the anterior adductor muscle. There is no mouth cavity, but a long, slender esophagus (fig. 3) leads directly to the stomach. The stomach (fig. 17, *stom*) is sacculated, and its walls contain several outpouchings where the ducts of the digestive diverticula open into it. The intestine arises at the ventral side of the stomach, and as it passes ventrally and forward it becomes gradually smaller. It is thrown into a coil (fig. 3) a short distance anterior to the middle of the foot, then proceeds posteriorly and upward to the heart. It runs through the ventricle and continues posteriorly along the dorsal body wall, over the posterior adductor muscle, and opens into the cloacal chamber through a small triangular papilla.

The entire alimentary canal is lined with tall columnar cells bearing rather long cilia. In the style sac, described below, the cilia are longer and much more numerous. The only parts of the digestive system which do not bear cilia are the ducts and tubules of the digestive diverticula.

*b. The digestive diverticula.* Surrounding the stomach and the esophagus, and completely filling the space between the esophagus and the intestinal coil, are the digestive diverticula (fig. 3), which are slightly brown in the living animal. These have been called liver and hepatopancreas, as well as digestive diverticula, but Yonge ('26: 703), in his paper on "The digestive diverticula of the lamellibranchs," says: "In some cases they constitute a gland; in others, including the Lamellibranchs, . . . ., their function is that of assimilation, and so they are most suitably designated digestive diverticula."

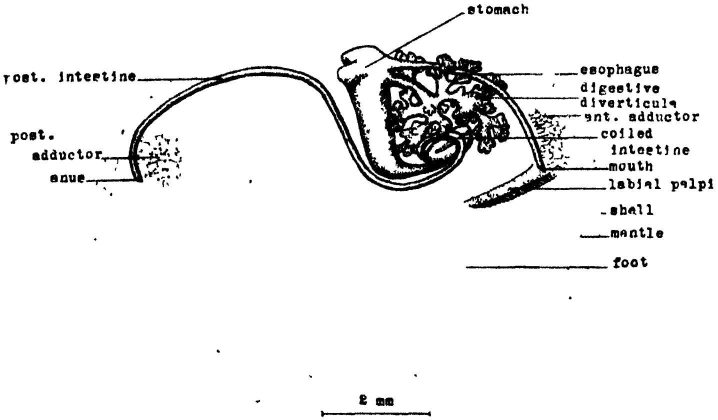

Fig. 3 Lateral view of the digestive system from the right side.

The diverticula form two groups of blind tubules which open into the stomach by means of common ducts. These ducts enter the stomach at its upper anterior side, just below the opening of the esophagus. The tubules are usually ovoid or nearly so in sections; they consist of a single layer of very tall cells about an elliptical lumen. The outer ends of the cells along the sides of the lumen are usually clear, as though their contents had been discharged, while the cells at the ends of the lumen usually have a granular cytoplasm which stains deeply. Figure 20 shows a section through a typical tubule.

*c. The crystalline style and style sac.* In all adult and in most of the young and juvenile animals sectioned, a crystalline style has been found in the intestine. Although this structure has often been studied and described, I have been able to find only casual references to its presence in the Sphaeriidae. Drew does not mention it. T. C. Nelson ('18: 53–112) gives a very good account of the crystalline style in certain marine and fresh-water mollusks. The fresh-water forms which he studied were Anodonta grandis, Lampsilis siliquoides, and L. anodontoides; the structures which he describes for Anodonta grandis are very similar to those found in S. notatum.

In very young and transparent specimens the style may often be seen in motion. Since the intestine is surrounded by the digestive diverticula, only the head of the style may be seen, in the lumen of the stomach. Sometimes it moves very slowly and steadily and sometimes very rapidly and erratically.

The cavity of the intestine is incompletely divided into two chambers by two poorly developed longitudinal folds in the epithelial lining. These folds, or typhlosoles, lie on the anterior and posterior sides in the dorsal end of the intestine, but on the dorsal and ventral sides in the lower part, due to the forward bend in the intestine just above the foot. Since the anterior typhlosole is the larger of the two, they will be referred to as the major and minor typhlosoles (fig. 18, *t ma* and *t mi*).

The left chamber of the intestine is the larger and contains the crystalline style. The smaller right chamber (fig. 18, *int*) is the intestine proper. The style sac (fig. 18, *st s*) is in direct communication with the intestine throughout its length, except for a very short, blind pouch at the base of the style sac, at the first sharp backward turn of the intestine as it enters the coil. The style sac is rather large at its upper end, but becomes gradually smaller until it terminates in the small blind pouch just mentioned.

The epithelium of the style sac is much heavier than that of the intestine proper. It is composed of tall, ciliated columnar cells, between which lie the glandular cells which secrete the style. The epithelium of the intestine proper is composed of a single layer of low ciliated cells. The epithelium of the style sac bears very numerous fine cilia which serve to keep the style in motion. These cilia are longer and much more numerous than the cilia in the rest of the digestive tract. They are confined to the style sac itself. The typhlosoles and the rest of the intestinal epithelium bear fewer and shorter cilia. Beyond the style sac the epithelium of the intestine is taller than in the intestine along the style sac, though not as tall as in the sac.

The epithelium of the style sac is thrown into a series of transverse folds, or ridges, which serve as bearings for the style, in exactly the same manner as Nelson ('18: 65–67) has described for Anodonta grandis. These folds are limited to the anterior wall of the sac.

The crystalline style (fig. 18, *st*) is a long, slender, hyaline rod made up of concentric layers of a clear, lightly staining substance, and a core of a more deeply staining substance. The style and style sac are nearly circular in cross-section; they have the greatest diameter nearest the stomach, and become gradually smaller until they end in the small blind pouch previously mentioned. The head of the style projects into the lumen of the stomach, and usually presents a frayed appearance (fig. 17, *st*). Figure 18 is a cross-section through the style sac and crystalline style a short distance below the stomach.

*d. The gastric shield.* The left side of the dorsal wall of the stomach is thrown into a fold, opposite the head of the style. This fold is devoid of cilia and carries on its surface a thick layer of a clear, hyaline substance (fig. 17, *g s*), which bears a prominent ventral projection immediately opposite the head of the style. The layer is continued over the epithelium of the wall of the stomach for a short distance surrounding the dorsal fold. Nelson ('18: 56) has proposed the name 'gastric shield' for this structure, as it evidently protects the gastric mucosa from the action of the head of the revolving style.

*e. The labial palpi.* The labial palpi (figs. 3 and 10) are long, slender, paired appendages which assist in bringing food to the mouth and in keeping foreign or injurious material away. There are two pairs of palpi, an inner shorter and an outer longer pair, which are attached to the body wall at the sides of the mouth and extend posteriorly and ventrally along the edges of the gills. Each palp consists of a core of loose connective tissue covered by a single layer of flat epithelium. The epithelium of the adjacent sides of the palpi is made up of large cuboidal, ciliated cells, and is thrown up into numerous strong transverse ridges. There is a deep groove (fig. 10, *gr*) which originates at the ventral edge of the head of the inner palp. This groove is continuous with the space between the two palps; it passes across the head of the inner palp and continues upward to the mouth. There is a flap of tissue which extends over the groove from the anterior side and practically converts it into a tube. The groove and the ciliated ridges on the adjacent sides of the palps evidently serve to pass food into the mouth after it has been brought forward by the water currents in the ciliated groove in the mantle.

### *5. The nervous system*

The nervous system consists of the three pairs of ganglia typical for the Acephala, their connectives and fibers, and two paired organs of special sense, the statocysts and osphradia.

*a. The ganglia and commissures.* The anterior, or cerebropleural ganglia (fig. 4), lie immediately posterior to the anterior adductor muscle, one on each side of the esophagus. In adult animals they lie about 0.7 mm. apart, and are connected by a short commissure that passes over the esophagus. They are nearly spherical and have a diameter of about 0.18 mm.

The pedal ganglia (fig. 4), lying in the foot, are the largest of the three pairs. They lie in the connective tissue immediately dorsal to the muscles of the foot, slightly anterior to the first bend in the intestine. They are ovoid in shape and are fused together by their adjacent sides (fig. 7, *ped ga*).

The visceral ganglia (fig. 17, *vis ga*) lie on the anterior side of the posterior adductor muscle, in the connective tissue below the posterior retractor pedis muscle. They are even more ovoid than the pedal ganglia, and their adjacent sides are also fused.

Table 1 gives the average measurements of the three pairs of ganglia in adult specimens.

TABLE 1

*Dimensions of the ganglia in S. notatum*

| *Ganglion* | *Length* *mm.* | *Width* *mm.* | *Depth* *mm.* |
|---|---|---|---|
| Cerebropleural, | 0.22–0.25 | 0.18–0.20 | 0.16–0.17 |
| Pedal, | 0.25–0.35 | 0.18–0.20 | 0.18–0.22 |
| Visceral, | 0.22–0.29 | 0.16–0.18 | 0.16–0.18 |

Each ganglion consists of a central mass of nerve fibers surrounded by a thick layer of nuclei. These nuclei are limited to the ganglia, none being found on the commissures and nerve fibers.

Connecting the cerebropleural ganglia with the pedal and visceral ganglia are two pairs of commissures. The cerebropedal commissure (fig. 17, *cp comm*) arises at the posterior-ventral side of the cerebropleural ganglion, passes posteriorly and ventrally through the anterior retractor pedis muscle, and follows along the mesial side of the muscle to the ganglion. The visceral commissure (fig. 16, *vis comm*) arises at the posterior side of the cerebropleural ganglion and passes posteriorly through the visceral mass near the body wall. It passes inside the posterior retractor pedis muscle and joins the visceral ganglion at its anterior end.

Besides these commissures, each ganglion gives off a number of nerve fibers to adjacent organs. Three pairs of nerves arise from the cerebropleural ganglia. The anterior pallial nerves (fig. 4) arise laterally from these ganglia, pass anteriorly along the sides of the anterior retractor pedis muscles and into the mantle; a pair of slender nerves have their origin at the anterior sides of the ganglia and pass to the anterior adductor muscle; another pair of slender nerves arise at the inner sides of the ganglia and pass to the esophagus.

The pedal ganglia give rise to a number of large nerve fibers which pass to all parts of the foot. Arising from the cerebropedal commissure at its junction with the pedal ganglion is a small nerve which passes directly to the statocyst. I have not been able to demonstrate nerve fibers in the statocyst, however.

The visceral ganglia give rise to a group of nerves very similar to the complex arising from the cerebropleural ganglia. The posterior pallial nerves (fig. 4) arise at the posterior sides of the ganglia and pass posteriorly and laterally over the roof of the cloacal chamber. There is a small accessory ganglion in the course of this nerve; from it a number of small nerves pass to the mantle, siphonal muscles and to the posterior adductor muscle. From the anterior side of the visceral ganglion arises the large branchial nerve (fig. 4), which follows along the partition between the epibranchial chambers of the inner and outer gills. At its origin is a small accessory ganglion, which is covered by a specialized patch of epithelium, the osphradium.

*b. The organs of special sense.* There are two pairs of highly differentiated organs of special sense, the statocysts and the osphradia. The generalized sense organs have not been included in this investigation.

*a.* The statocysts and statoliths. The statocysts (fig. 17, *stat*) are small, paired ovoid organs having to do with the maintenance of equilibrium. They are suspended in connective tissue about 0.1 mm. anterior to the pedal ganglia immediately above the anterior retractor pedis muscles, against which they sometimes lie. In the adult animal they are about 0.1 mm. long and 0.065 mm. in diameter.

Each statocyst (fig. 15) consists of a heavy outer fibrous capsule, lined with a single layer of large vesicular cells having large spherical nuclei. Drew ('96: 178) states that he was unable to find cilia in the statocysts of S. sulcatum, and suggests that his fixation may have been at fault. Pelseneer ('06: 238) states that cilia may be absent in forms in which an otolith is present. Kellogg ('92: 411) states that cilia are not found in the statocysts of Yoldia. I believe that cilia are not present in the statocysts of S. notatum, for though they have been conspicuous in other situations in the sections used for the study of the statocysts, they do not appear in the statocysts.

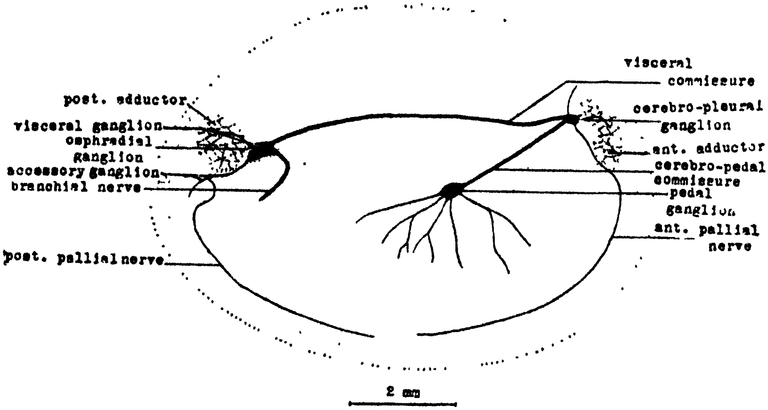

Fig. 4 Lateral view of the nervous system from the right side.

The statolith (fig. 15, *stl*) is a small spherical concretion lying in the cavity of the statocyst. It is about 0.019 mm. in diameter and has a slightly wrinkled appearance in the sections. It is not affected by most stains, but stains deeply with iron hematoxylin.

Pelseneer ('06: 238) states that the statocysts of the Acephala are supplied with a small nerve from the cerebropleural commissure. Drew ('96: 177–178) states that the innervation is difficult to demonstrate in S. corneum. As stated in the section on the ganglia, I have found a small nerve which passes from the junction of the cerebropleural commissure with the pedal ganglion directly to the statocyst, but I have not been able to demonstrate nerve fibers within the statocyst.

*b.* The osphradia. The osphradia are small paired areas of specialized epithelium lying immediately below the osphradial ganglia (fig. 16, *os ga*) and extending a short distance forward along the course of the branchial nerve. Each area is oblong in shape, being about 0.05 mm. wide and 0.25 mm. long.

This organ is found in most Acephala, and Pelseneer ('06: 236–237) suggests that it may serve to test the respiratory fluids. While this view is held by most workers on the Mollusca, its inadequacy becomes obvious when the location of the osphradium is observed. In S. notatum, as previously stated, the osphradium lies in the roof of the cloacal chamber along the course of the branchial nerve, where it is bathed only by that current of water which has already passed through the gills. It is evident that an organ placed in such a position cannot function effectively to test the respiratory fluids. In so far as I have been able to determine, no experimental work has been carried on to determine the function of the osphradia, yet writers continue to quote the assumption that it serves to test the respiratory fluids. The function of the osphradia of the Acephala remains to be demonstrated experimentally.

### *6. The circulatory system*

The circulatory system consists of a single ventricle, two auricles, and a number of blood vessels, including the large blood spaces in the gills (fig. 19, *bls*).

Immediately posterior to the dorsal end of the stomach, and surrounding the posterior intestine, lies the thin-walled pericardial cavity (fig. 17, *p c*). The pericardial cavity contains the single median ventricle and the paired auricles. The ventricle (fig. 17, *vent*) is an oval organ surrounding the intestine. Its walls are somewhat heavier than the walls of the other blood vessels, due to the presence of two thin layers of muscle fibers, an inner circular, and an outer longitudinal layer. The auricles lie at the sides of the ventricle, into which they open just below the middle of each side. The auricles extend posteriorly and ventrally below the kidneys and are continuous with the blood spaces in the gills.

Arising from the ventricle are two large trunk arteries, an anterior and a posterior. The opening into each is guarded by a valve (fig. 17, *va*), consisting of a single flap of tissue. The anterior artery (fig. 5) passes over the dorsal side of the stomach, then across the right side of the esophagus, which it follows anteriorly, supplying the digestive diverticula and the coiled intestine. At the level of the dorsal end of the anterior adductor muscle it gives off a branch which passes in front of the muscle and divides into branches supplying the mantle. The main branch continues forward along the esophagus a short distance, then turns and passes ventrally and posteriorly into the floor, where it sends branches into all parts. Only a few branches in the foot are well differentiated, the rest are continuous with the numerous spaces permeating the whole muscle mass.

Immediately upon leaving the ventricle, the anterior artery divides into two branches; the main branch passes forward as described and the other (fig. 5) passes ventrally along the posterior side of the stomach and intestine and forward into the intestinal coil.

The large posterior artery surrounds the posterior intestine. It passes posteriorly over the posterior adductor muscle and there divides to supply the posterior parts of the mantle.

The walls of the blood vessels, except those of the ventricle, consist of a single layer of tissue, with many small nuclei scattered throughout.

In very young and transparent specimens the pulsations of the heart may be observed through the shell. The rate of the beat varies widely, but in flowing water the rate is about

90 to 100 per minute. In the protruded foot of young specimens taken from the brood pouch, the colorless blood may be seen moving back and forth by a series of surges. The forward movements are of longer duration than the backward movements, which are due to the contractions of the foot.

### 7. *The excretory system*

The excretory system consists of a pair of very much coiled and sacculated tubules, which constitute the organs of Bojanus. These tubules occupy the space between the posterior adductor muscle and the pericardium (fig. 17, *kd t*).

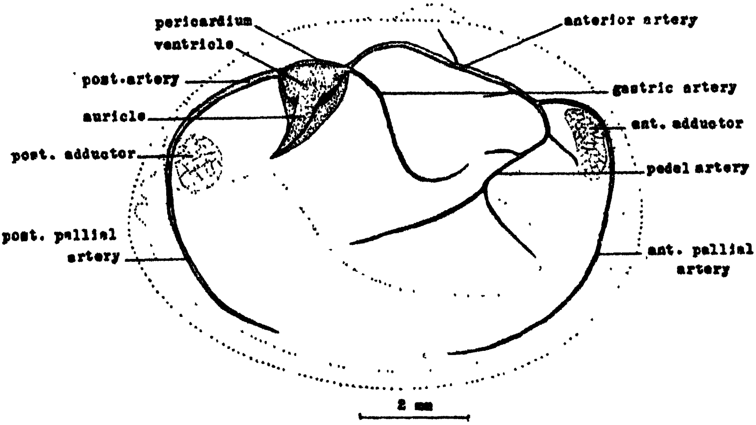

Fig. 5 Lateral view of the circulatory system from the right side.

The kidney tubules are open at both ends. At one end they open into the posterior ventral angle of the pericardial cavity by means of a small ciliated duct about 0.5 mm. long (fig. 13, *c d*). At the other end they open into the cloacal chamber through a non-ciliated tube which is joined near its opening by the genital duct (fig. 6, *2*).

The tubules (fig. 13, *kd t*) are made up of a single layer of small cuboidal cells lying upon a thin external basement membrane. These cells contain large vacuoles in their free ends and many fine granules near the basal membrane. With the exception of the duct opening into the pericardial cavity, the kidney epithelium is not ciliated. This duct is lined with deeply staining, flattened cells which bear many long cilia. The free ends of these cilia are always directed inward, indicating that the direction of their action is from the pericardial cavity into the kidney. There is an abrupt transition from the low ciliated epithelium of this duct to the epithelium of the much-coiled tubule which it joins near the center of the kidney. There is no direct communication between the groups of tubules of each side.

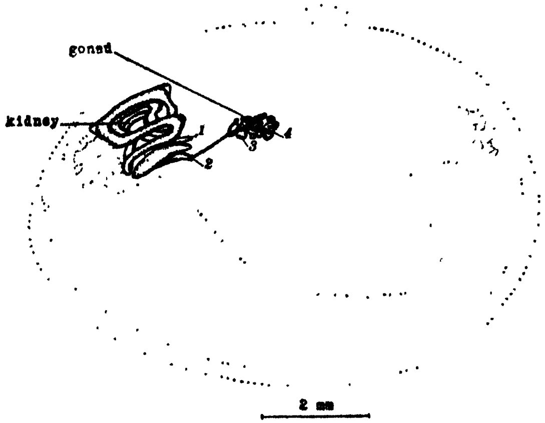

Fig. 6 Lateral view of the excretory and reproductive systems. *1*, ciliated duct opening into pericardial cavity; *2*, junction of the genital duct with kidney duct opening into cloacal chamber; *3*, ova-producing follicles; *4*, sperm producing follicles.

### *8. The reproductive system*

This species, as is the case with all the Sphaeriidae, is hermaphroditic, having both sperm- and ova-producing follicles in the gonads. As both Drew and Gilmore have accurately described the reproductive organs in members of this genus, only a general description will be given here.

The gonads consist of a number of spherical or ovoid follicles (fig. 6, *3* and *4*) grouped about a common duct, into which they open by mean's of separate ducts. The sperm-producing follicles are anterior to the ova-producing follicles and are more numerous and somewhat smaller. The follicles are usually filled with masses of fusiform spermatozoa. I have been unable to find tails upon any of the spermatozoa. The ova-producing follicles are slightly larger than the sperm follicles, and usually contain from four to eight mature ova (fig. 14, *m o*) besides a number of immature ones, during the fall and winter. During the summer both types of follicles are almost devoid of germ cells and are considerably shrunken.

Each follicle is supplied with a duct leading to the common duct, which opens into the cloacal chamber in common with the duct of the kidney. The ducts consist of a single layer of closely packed cells which stain deeply. These cells are not ciliated.

## THE LIFE-HISTORY

Very little has been published on the life-history of the American Sphaeriidae. Thiel ('27), as previously mentioned, has recently published the results of work carried on at Hamburg, Germany, on the life-history of Sphaerium corneum.

The young develop in brood pouches formed in the upper parts of the inner lamellae of the inner gills and are expelled through the exhalent siphon. When born, the young of this species are from 2.5 to 4 mm. long.

The routine examination of the specimens collected for this study revealed the fact that individuals under 9 mm. in length were sexually immature. Therefore, in the following experiments, those between 9 and 14 mm. were selected. Several aquaria with twenty-five to thirty adults in each were kept in a tank of running water. These were examined periodically for young sphaeriids, which were removed when found. Under laboratory conditions, it was hard to keep the animals alive, making it impossible to get a long series of observations upon a single group and necessitating repeated renewals of the stock from field collections.

Many adults were found in the drainage ditch from September to the end of November. No animals longer than 7 mm. were found from then until early in the spring, when full-grown animals were again found. Conditions in the drainage ditch have been very unusual during the past year. There have been many protracted periods of high water, with a large amount of wash resulting, following heavy rains. During May and June, following periods of high water, no adult animals were found. Early in July, following another period of high water, many adults were found.

Very few young animals were found in the aquaria during the fall and winter. During the month of April, ten young animals were taken from a single aquarium. The animals collected in July were found to be giving birth to many young. Twelve were found in each aquarium the first week, and six the second. During the first week of August, it was observed that the full-grown animals were giving birth to but few young, while the young adults, from 9 to 11 mm. in length, were giving birth to quite a few.

From these observations it is evident that reproduction is at the maximum during the summer months and that very few young are born during the winter.

The condition of the reproductive organs in animals killed at various times during the year indicates that fertilization probably takes place in the late fall and that the young are incubated in the brood pouches during the winter. As has been previously noted, in animals killed early in July the germ follicles are much shrunken and very nearly empty. In animals killed during the winter the follicles are extensive and well filled. The brood pouches contain fewer and smaller embryos in July than in the winter.

## SUMMARY

Sphaerium notatum is very similar in its general organization to the larger fresh-water Acephala. The various organ systems are described and figured in detail. The histology of many of the organs has been considered. The crystalline style and style sac commonly found in other Acephala are also found in this species. A pair of slender muscles supporting the gills, not hitherto described, have been found. Attention is called to the fact that the function commonly ascribed to the osphradia is incompatible with their position in the roof of the cloacal chamber. Reproduction is evidently at the peak during the summer months.

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# PLATES

## ABBREVIATIONS

*ant a*, anterior artery
*ant add*, anterior adductor muscle
*a r p*, anterior retractor pedis muscle
*aur*, auricle
*bls*, blood space
*blv*, blood vessel
*br po*, brood pouch
*by gl*, vestige of byssal gland
*cap*, fibrous capsule
*ccc*, ciliated corner cells
*c d*, ciliated duct
*c gr*, ciliated groove in the mantle
*ch r*, chitinous rod
*cl*, median cleft
*clo*, cloacal chamber
*cp comm*, cerebropedal commissure
*c r*, ciliated ridge
*c t*, connective tissue
*dig div*, digestive diverticula
*emb*, embryo
*ep*, epidermis
*ep gl*, epidermal gland
*esoph*, esophagus
*fil*, gill filament
*go*, gonad
*gr*, groove leading to the mouth
*g s*, gastric shield
*i g*, inner gill
*i l*, inner lamella
*in j*, interfilamentary junction
*int*, intestine
*i p*, inner palp
*kd t*, kidney tubules
*lcc*, lateral ciliated cells
*lev branch*, levator branchiarum muscle
*ll pl m*, longitudinal pallial muscles
*m o*, mature ovum
*mth*, mouth
*mtl*, mantle
*mu*, mucous glands in the foot
*ob pl m*, oblique pallial muscles
*o g*, outer gill
*o f*, ovarian follicle
*o l*, outer lamella
*o p*, outer palp
*os ga*, osphradial ganglion
*osph*, osphradium
*p c*, pericardial cavity
*ped ga*, pedal ganglia
*post a*, posterior artery
*post add*, posterior adductor
*post int*, posterior intestine
*p r p*, posterior retractor pedis muscle
*se comm*, supra-esophageal commissure
*s f*, sperm follicle
*st*, crystalline style
*stat*, statocyst
*stl*, statolith
*stom*, stomach
*st s*, style sac
*t ma*, major typhlosole
*t mi*, minor typhlosole
*va*, valve in artery
*vent*, ventricle
*vis comm*, visceral commissure
*vis ga*, visceral ganglia
*wt*, water tube

## PLATE 1

### EXPLANATION OF FIGURES

7 Cross-section of an adult animal through the stomach and pedal ganglia.

8 Cross-section of an adult animal through the ventricle.

9 Cross-section of the ventral edge of the mantle.

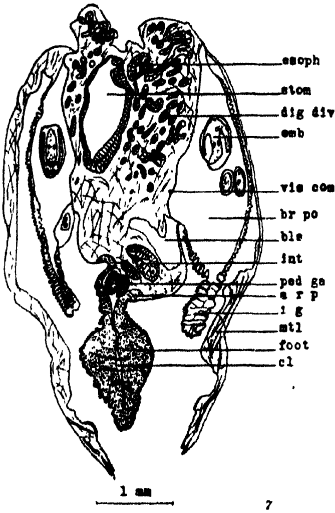
10 Diagrammatic ventral view of the groove passing from the labial palps to the mouth.

11 Sagittal section through the edge of the foot, showing the mucus-secreting cells and the thin membrane covering the external epithelium.

12 Sagittal section of the wall of the esophagus.

13 Sagittal section through the kidney, showing the opening of the ciliated duct into the pericardial cavity.

14 Section through the gonad, showing both ova- and sperm-producing follicles.



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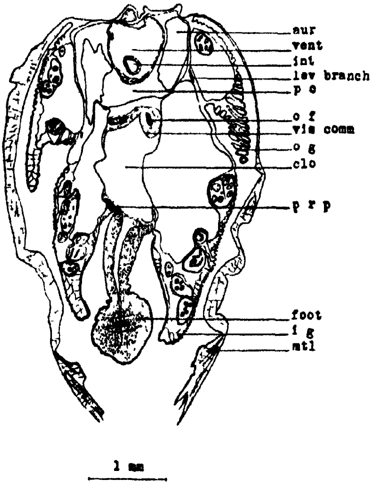

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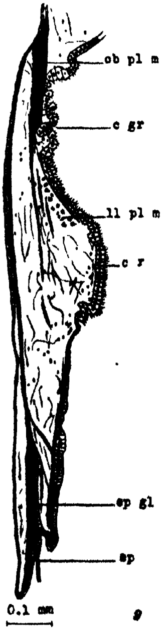

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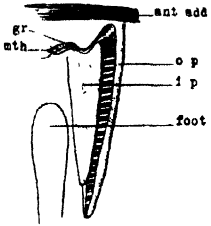

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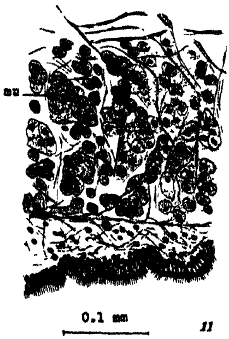

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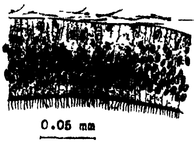

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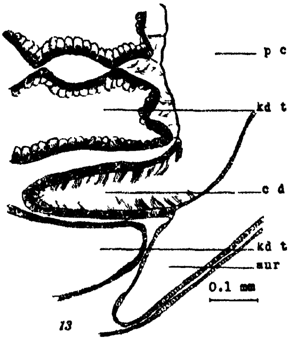

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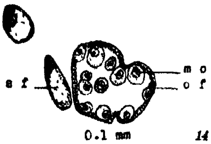

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## PLATE 2

### EXPLANATION OF FIGURES

15 Sagittal section through the statocyst, showing the statolith in place.

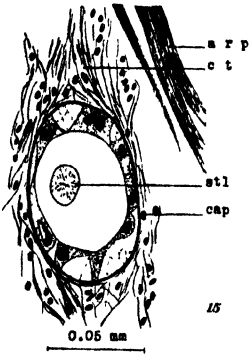
16 Sagittal section of visceral and osphradial ganglia and the osphradium.

17 Sagittal section of entire animal through foot and stomach.

18 Transverse section of intestine and crystalline style, a short distance below stomach.

19 Frontal section through the inner gill.

20 Section through a typical tubule of digestive diverticula.



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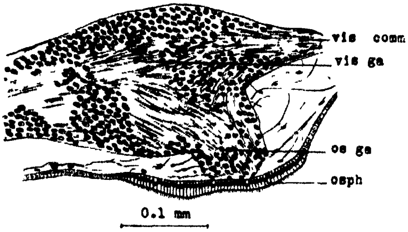

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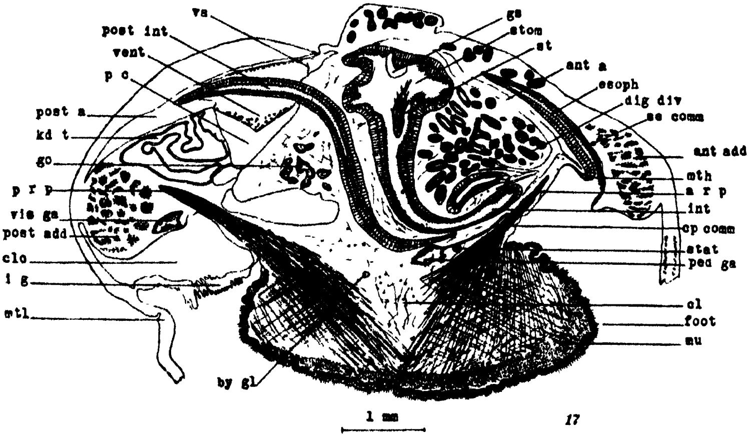

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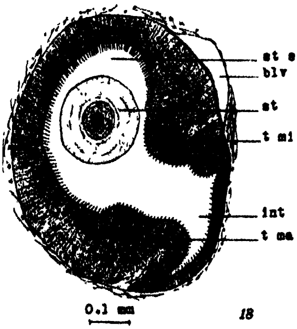

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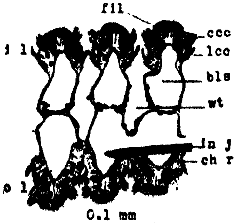

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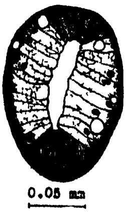

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# THE MORPHOLOGY AND PHYSIOLOGY OF THE HEPATOPANCREAS OF CAMBARUS VIRILIS[1]

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SEVENTEEN FIGURES

AUTHOR'S ABSTRACT

The relation of the hepatopancreas to the pyloric stomach and its physiologic histology were studied. The organ consists of two lobes, united ventrally, which lie along the cardiac and pyloric stomachs and the intestine Primary and secondary lobulations (acini) are present, and the entire organ is a system of anastomosing tubules (lumina). The tubules progressively converge and form a duct in each lobe which leads ventrolaterally into the pyloric stomach.

Each acinus consists of a single layer of hepatic and pancreatic cells supported by a basement membrane, and the interacinar spaces are the seat of fibro-elastic tissue and phagocytic cells The supporting tissue is laminar with that of the stomach The arterial capillaries, composed of endothelial cells, lie in the interacinar spaces. The blood supply is by way of the basement membrane to the cells.

The hepatic cells of specimens collected in September bear little fat, cells of June specimens are laden with fat. Glycogen is deposited in the hepatic cells, there is no difference between the quantity found in September and in June. The hepatic cells probably contain biliverdin Chemical analysis indicates the presence of trypsin, amylase, and lipase

A review of the literature and a discussion of homologies with reference to the vertebrate liver are given.

## INTRODUCTION

The present paper is one of a series, which deals with the liver or hepatopancreas of the invertebrates. At the beginning of the investigation in September, 1926, it was proposed that this organ in at least one member of each of the following phyla be studied, with particular reference to the cellular structure and the deposition of fat and glycogen: Coelhelminthes, Arthropoda, Echinodermata, Mollusca, and Chordata (prochordate). One of the common fresh-water decapods, Cambarus virilis, has been employed as a point of departure in the study, largely because of its availability and the morphologic prominence of the gland. Other forms used in this series are: Nereis virens, Asterias vulgaris, Loligo pealii, Melanoplus femur-rubrum, and Balanoglossus aurantiacus.

The results of the study on Cambarus virilis fall into these divisions: 1) general structure; 2) the structure of the acinus, the unit of the organ; 3) the blood supply; 4) the supporting tissue, and, 5) the deposition of fat and glycogen. I shall refer to the organ of Cambarus virilis as the hepatopancreas, the reason for which will be made clear.

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[1] From the Division of Experimental Surgery and Pathology.

## REVIEW OF THE LITERATURE

The literature on the liver or hepatopancreas of the invertebrates naturally is concerned largely with histology and functions and a comparison of the latter with those of the vertebrates. I shall review here chiefly such parts as are concerned with the structure of the gland and the deposition of fat and glycogen in the cells of the organ.

Investigations have been made on the digestive glands of members of at least these major groups: Trochelminthes, Annelida, Arthropoda, Mollusca, Echinodermata, and the Cephalochordata. It seems, therefore, that, so far as previous work is concerned, no hepatic gland has been described in any group of animals simpler in organization than the segmented worms. However, L. H. Hyman[2] is of the opinion that although in Planaria "there are no definite digestive or multicellular glands connected with the digestive tract . . . . the lining epithelium of the digestive tract appears to have all the functions of a digestive gland. . . ." These cells "store food in the form of fat and protein but not glycogen. . . . From these characteristics[3] it would seem to me that these epithelial cells of Planaria have much in common with vertebrate hepatic cells. It is probable that the entodermal cells of coelenterates and sponges have the same properties as those of planarians." Although there is no digestive apparatus in the Cestoda, determinations have been made on the deposition of glycogen.

Weinland ('01, '01 a, '04), determining the glycogen content of Taenia expansa, Ascaris lumbricoides, and Ascaris mystax on the basis of the dry residue (by weight) of the worm, found the quantity in the first to be from 15 to 47 per cent, and in the second and third, from 20 to 34 per cent. Busch ('05) reviewed his work on the determination of glycogen in Taenia and in nematodes and showed figures in color to indicate deposition. He found in Taenia that accumulation of glycogen in the parenchyma begins as soon as the eggs appear, and that, when the increased volume of the uterus produces flattening of the parenchyma, the quantity of glycogen gradually decreases. Glycogen also accumulates in the muscles of the bothria. The deposition of glycogen in nematodes occurs especially in the sacciform appendixes of the longitudinal muscles, in the hypoderm, in the epithelium of the intestine, and in the germ cells.

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[2] Personal communication.

[3] I have omitted production of enzymes, deaminization, and so forth.

Cuénot ('98), in one of his classic works on the physiology of the invertebrates, found that in Lumbricus hurculeus, Allolobophora terrestris, Lumbriculus variegatus, Tubifex rivulorum, Naïs elinguis, and Branchiobdella parasita glycogen is "le produit de reserve habituel," that is found in the cells of the peritoneal epithelium (which, he says, are identical in form and function to 'cellules de Leydig' in Mollusca and Decapoda), in the cells of the metanephridia, in the cells of the septa, and, in Tubifex, around the ventral blood vessel. During the feeding season these cells form, notably in Lumbricus, enormous masses on the septum; when food is less plentiful, the masses are reduced to almost nothing. Glycogen also is found, but not in large quantities in the chlorogogue cells and in the amebocytes of the body fluid of Lumbricus and Tubifex. Cuénot never found glycogen in the muscles. Instead of glycogen he often found fat in Phreoryctes, both in the peritoneal and chlorogogue cells. Fat also was found in small quantities in Branchiobdella.

Stone ('97) used the pyloric cecum of about twenty living starfish in a test for glycogen, for, she says, "in determining whether the function of the pyloric ceca of the starfish is more similar to that of the liver or to that of the pancreas of higher animals, one of the most reliable criteria would be the existence or non-existence of glycogen in the organ." Using Pflüger's ('94) method for glycogen determination, she concluded that, "the total amount of glycogen in the glands of these twenty starfishes must, therefore, have been very small, thus indicating that these organs do not possess a function characteristic of the true livers of the higher animals." A test for glucose, the antecedent of glycogen, likewise was negative.

The literature on this organ of the Crustacea is abundant and varied in conclusions. Huxley ('80) stated that each lobe of the liver of the crayfish, Astacus fluviatilis, "consists of an immense number of short tubes, or ceca, which are closed at one end, but open at the other into a general conduit, which is termed their duct. . . . The liver may be regarded as a much-divided side-pouch of the mid-gut." Schneider ('02) wrote of the liver of Astacus fluviatilis as a 'paarige Mitteldrüse' which has 'einen tubulösen Bau,' but his description of the cellular structure was more detailed than that of Huxley. He considered nourishing, excretory, and glandular (ferment) cells. Dahlgren and Kepner ('08) regarded the crayfish liver "as an example of the more highly specialized digestive tissues of the Arthropoda." Guieysse ('07) found that the cells of the hepatopancreas of the Crustacea mentioned later are of the intestinal type.

Concerning the functions of the liver in the crayfish and related forms of Decapoda, and in the Isopoda, Amphipoda, and Copepoda, a number of investigators are in disagreement. Guieysse ('07), working on Calanus, Palinurus, Palaemon, Carcinus, Portunus, Squilla, Helleria, and Orchestia, concluded that the organ called the hepatopancreas is a group of diverticula of the midintestine, and not a gland with the unique function of pouring its products into the stomach. Deflandre ('05) attributed to the liver of the Decapoda, digestive, excretory, and absorptive functions, as well as 'une fonction d'arrêt.' The latter she demonstrated by injection experiments, recovering the coloring matter in the hepatic cells, and by feeding of arsenic and later finding the poison almost exclusively in the hepatic cells. The same author found that the reserve of fat is more abundant in the liver of Astacus fluviatilis in April than in November. Abelous and Billard ('97) ascribed an anticoagulating function to the liquid exuded from the liver of the crayfish comparable to the effect produced by the extract from the leech. Huxley ('80) ascribed the yellow color of the secretion of the liver of Astacus fluviatilis to globules of fat. Hoppe-Seyler ('77) found that there is no more glycogen in the liver of the crayfish than in any other organ, and none of the true components of bile. Perrier ('93) stated that there is no trace of biliary products in the pyloric glands of the crayfish and that these glands do not contain glycogen. MacMunn ('99) found in the liver a pigment, which he termed 'entero-chlorophylle.' Cuénot ('98), whose opinion was based on the results of his work on the Orthoptera, concluded that the hepatopancreas absorbs peptones and sugars. He further stated that the liver of the Decapoda possesses digestive, biliary, and excretory functions, the product of secretion normally eliminated being represented by the pigment of 'les cellules à ferment.'

McMurrich ('98), working on Armadillidium, found that the liver is represented by three pairs of ceca extending parallel to the digestive tube. Having fed cochineal, he observed that the cells of the intestine absorbed none, but the hepatic cecum was filled with it.

Patrick ('26) described the hepatopancreas of Ligia oceanicus as consisting "of three pairs of tubules which extend from the pyloric end of the stomach to the posterior end of the abdomen. The tubules open into the pylorus." The cells are of two kinds: large, irregular, columnar cells, and, interspersed among these, smaller, oval or wedge-shaped cells, "also attached to the basement membrane, fewer in number and shorter."

Several authors have described the hepatic organ in various species of the Arachnida. Dugès (in Cuvier's Regne Animal) described four pairs of diverticula in the lower part of the alimentary canal of Mygale caementaria, and these diverticula were interpreted by Biedermann ('11) as an hepatic organ. Bertkau, according to the latter, believed that he demonstrated the presence of glycogen in the fusiform cells of the diverticula of Amaurobius ferox, and, in well-fed individuals, these cells contained fat. Berlese ('96) ascribed to the liver of Trombidium the power to store fat and produce 'peptonization.'

Morse ('02), in his observations on living Brachiopoda, stated that all species of this group possess "stomachal glands usually known as the liver." Young stages of Disconisca, Terebratulina, and Hemithyris have tuft-like masses of ceca, which "seem as fully charged with diatoms and other food material as the stomach itself." In some species of the Lingulidae the glands are lobular or blunt masses. Morse concluded (without histologic study) that the stomachal glands are "extensions of the absorbent surface of the digestive tract, and are probably hepato-pancreatic in their nature."

Investigations have been made on the liver of at least four classes of the Mollusca, but this organ in the Cephalopoda probably is better known than that in the Amphineura, Acephala, or Gasteropoda. Schneider ('02) described the liver of Chiton siculus as acinous and composed of cylindrical and broad-based cells; the latter never reach the lumen of the acini. Both types of cells are granular. Biedermann ('11) found that there are two types of liver in the Amphineura: in Chaetoderma one diverticulum leads into the middle portion of the intestine, while the entire intestine of Neomenia and Proneomenia is beset with perpendicular diverticula. The latter condition is closely simulated in Tergipes and Aeolis (of the nudibranchs), and was termed diffuse by Lang ('02).

Among the livers of the gasteropods, by far the most attention has been devoted to Helix pomatia. Schneider ('02) described the organ as voluminous, three-lobed, ramifying, and tubular, consisting of hepatic, excretory, and calcium cells. The excretory cells are similar to those of Astacus. Fischer ('92) and Yung ('87) also described the organ, the latter as follows: four lobes, which are lobulate; a follicular gland, extremely ramified; follicles possessing convergent canals. Biedermann and Moritz ('98) described resorptive and excretory cells; the former were called hepatic cells by Barfurth ('83), granular cells by Frenzel ('86). Barfurth calls the excretory cells (of Biedermann and Moritz) ferment cells. Biedermann and Moritz, Cuénot ('00), Levy ('90), and Krukenberg ('82) found glycogen in the hepatic (resorptive) cells. The first-mentioned authors also found fat. Levy found that there was no difference in the quantity of glycogen present in summer and winter. Barfurth found glycogen in the ferment cells, and this author and Yung ('87) found that the glycogen content in the liver of snails is reduced to almost nothing by starving for two or three weeks. Barfurth ('85) also found glycogen in the liver of Limax variegatus. Krukenberg found glycogen in the liver of Arion ater. Several of these authors also have described the secretion from the livers of Helix and Limax.

The studies on the digestive glands of the cephalopods have been chiefly from the aspect of enzymes. However, the structure of the glands and the deposition of glycogen and fat also have received the attention of a number of investigators. Bourquelot ('82) pointed out that the pancreas of the cuttlefish is isolated from the liver, and Bauer ('09) found that, in Eledone moschata, the pancreas is attached to the surface of the broad end of the ovoid, acinous liver. The hepatic and pancreatic ducts are separate and distinct, and both empty into the spiral, cecal outpocketings of the intestine. Frenzel ('86) found two kinds of cells in the livers of Octopus vulgaris and Sepia officinalis, ferment and calcium cells, and Cuénot ('00) designated the two kinds as 'cellules vacuolaires' and 'cellules à boules.' Bourquelot ('85) found that the livers of Sepia, Octopus, and Loligo contain glycogen. The ferment cells, according to Frenzel, contain brownish-yellow granules, which he interpreted as fat. Enriques ('01) agreed with Frenzel that carbohydrates are stored in the cells of the liver of the cephalopods. Cuénot ('07) found fat in the form of droplets in the 'cellules à boules' of Sepia officinalis.

## MATERIALS AND METHODS

Specimens of Cambarus virilis were collected from a stream about eight miles from Rochester, Minnesota, during September, 1926, and June, 1927.

The hepatopancreas and the pyloric stomach were removed intact from several specimens and fixed in various fluids: Zenker's, Gilson's, Bouin's, Flemming's, 10 per cent formalin, and 100 per cent alcohol. For general study, Zenker's fluid was followed by Mallory's connective-tissue stain, methylene blue and eosin, and Delafield's hematoxylin and eosin. Mallory's stain is especially desirable for demonstrating the fibro-elastic tissue of the interacinar spaces.

For the demonstration of glycogen, tissue was fixed in 100 per cent alcohol, embedded in celloidin, stained in Best's carmine, and differentiated in alum hematoxylin. Frozen sections are essential to the demonstration of fat. The tissue was fixed in 10 per cent formalin for from twenty-four hours to several weeks, stained in scarlet red, and differentiated in alum hematoxylin.

The hepatic artery was conveniently filled with India ink and the blood supply of the hepatopancreas determined by filling the pericardial sinus with ink, either by removal of a part of the carapace or by the insertion of a hypodermic needle through the carapace. The best results, however, were obtained by direct injection into the heart by means of a capillary cannula.

## MORPHOLOGY

### *Gross morphology*

The hepatopancreas of Cambarus virilis consists of two identical lobes which lie along the gastro-intestinal tract in the dorsal part of the cephalothorax and, to a slight extent, in the anterior end of the abdomen. Viewed from above, the lobes extend along the cardiac stomach, almost entirely obscure the pyloric stomach, and extend several millimeters (depending on the length of the specimen) on each side of the intestine. Apparently, the lobes are completely separate, but gross and microscopic examination reveal that they are connected one to the other on the ventral surface of the pyloric stomach into which they empty (figs. 11 and 16).

The size of the organ naturally varies according to the size of the animal and this also is true of the portions of the lobes anterior and posterior to the ducts. In specimens from three to five months old, the length of the organ is from 1 to 1.5 cm. and the ratio of the anterior to the posterior part is about 4:5; in specimens one year old, about 7 cm. long, the ratio is about 2:3.

Each lobe is primarily and secondarily lobulate, the latter condition being observable microscopically. A fissure diagonal to the transverse, a second sagittal fissure slightly diagonal to the longitudinal, and a third frontal fissure, which is parallel to the longitudinal axis, superficially divide each lobe into four parts. The second and third lobulations in each lobe form prominent dorsal projections, which are in contact one with the other, dorsal to the pyloric stomach. I term these lobulations 'superficial' for, although they constantly are present in this species, the corresponding fissures are not deeply accentuated (fig. 16).

The color of the organ varies and probably is influenced to a certain extent by conditions that change with the season. In specimens which were collected in September the hepatopancreas was predominantly dull yellow, while in those collected in June it was usually green or greenish yellow. The color of the contained secretion corresponds to the color of the organ. The organs of specimens collected in the autumn are firmer and less likely to disintegrate than those collected in the summer.

## *Histology*

A membrane serves as a covering for the organ; it is thicker on the anterior and posterior portions than in the middle region. The secondary lobulations do not correspond strictly to the primary lobulations. Each lobe of the organ is a network of lobulations, which I shall call acini (fig. 11). Each acinus delimits an irregular lumen, which may be termed a tubule (fig. 14). Between the acini are definite spaces throughout the organ, and in these spaces are found the blood vessels and the reticulum of connective tissue (figs. 1, 2, and 11).

The arrangement of the acini is such that the peripheral units anastomose one with another, and this arrangement is continued and accentuated toward the hiluses. The end result simply is that all of the tubules of the organ gradually lead directly or indirectly into a common tubule which serves as a duct (figs. 1 and 11); this empties ventrally into the pyloric stomach. The similarity between the duct and the ordinary tubules of the acini should be emphasized. There are no real differences between the basement membrane of the duct, its cells, or the form of its lumen and the corresponding parts of any acinus in the organ. The duct is attached to the transitional epithelium of the pyloric stomach, and there is more fibro-elastic tissue in the region of attachment than around any other one unit of the organ (figs. 1, 2, and 11).

The form of the acini in cross-section and longitudinal section varies widely. In general, however, the cross-sections are round to ellipsoid exteriorly and an irregular lumen is present (figs. 1, 11, and 14). The smallest diameters of the acini are from 80 to 150 μ in the central region and the greatest diameters from 100 to 200 μ. Longitudinally, the acini follow a devious course and there is no good means of describing them except to say that, in general, they vary from straight to slightly curved and are pressed into slight aberrations by the surrounding elements. That is to say, the sur-

### ABBREVIATIONS FOR ALL FIGURES

*a*, artery
*ac*, acinus
*b m*, basement membrane
*e w*, endothelial wall
*f e c*, fibro-elastic cells
*f e t*, fibro-elastic tissue
*h p d*, hepatopancreatic duct
*l p*, large phagocytes
*p c*, pancreatic cells
*p s*, pyloric stomach
*s p*, small phagocytes
*t e*, transitional epithelium

face of an acinus may be indented at several points by pressure of other acini. The length varies even more than the diameter, ranging from 0.2 to 0.8 mm.

The hepatic cells, the pancreatic cells, and the inner and outer membranes are the chief elements in the structure of the acini. Both kinds of cells stain well in most of the general stains after fixation in almost any of the common fixatives. However, the use of Mallory's triple connective-tissue stain proved a convenient means of studying the cells and the membranes: the two kinds of cells are stained by the acid

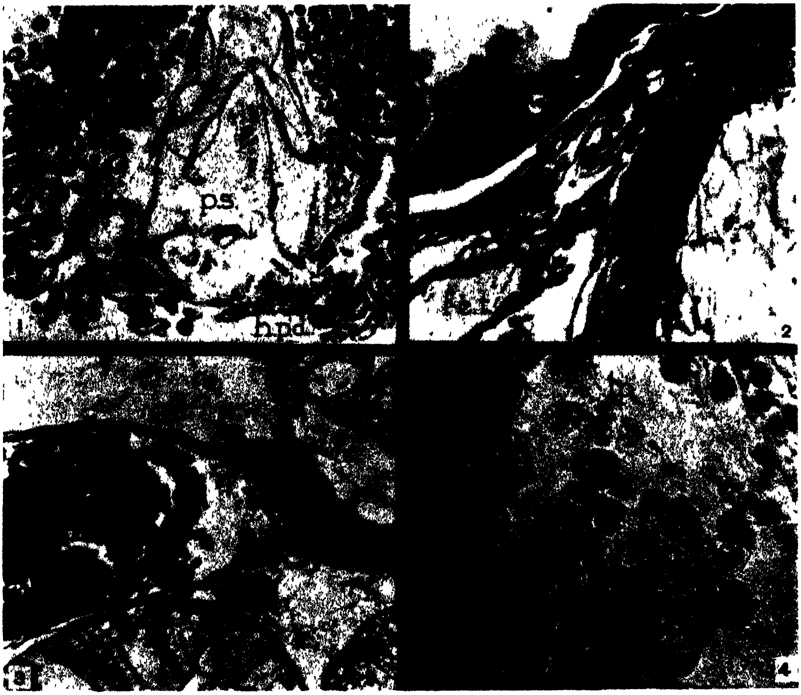

**Fig. 1** Cross-section of the hepatopancreas in the region of the ducts. × 20.
**Fig. 2** Cross-section in the region of the ducts: fibro-elastic tissue. × 20.
**Fig. 3** Cross-section of an injected artery in an interacinar space. × 120.
**Fig. 4** An interacinar space: fibro-elastic tissue and phagocytes. × 350.

fuchsin and the membranes by the anilin blue. Delafield's hematoxylin and eosin afford excellent differentiation, especially of the fibrillar condition in the hepatic cells of specimens collected in June, but the delicate blue staining of membranes by Mallory's stain is an advantage in the determination of their presence.

The hepatic cells are of the columnar epithelial type, and this typical condition may be conveniently observed in sections cut precisely through the longitudinal axes of the acini (pl. 2). Naturally, some of the cells are modified in form by pressure and a large number of squamous cells are present, especially at points of curvature in the lumen and on the surface of the acini. The attenuated end of each squamous cell is always pointed in the opposite direction from the convex surface of such curvatures.

The dimensions of the hepatic cells vary considerably: the long axes range from 35 to 50 μ and the short axes from 10 to 15 μ. The nuclei, which are typically round and possess a definite and clear membrane, measure up to 13 μ. Each cell contains one or more nuclei, and, if only one, it is remote from the lumen of the acinus. Each nucleus bears an intensely staining nucleolus. All of the cells are granular, and the majority of them possess pigmented inclusions (probably biliverdin), which, if small, lie adjacent to the nucleus. In some cells, which bear large deposits of biliverdin, the nucleus (near the membrane) and the membrane are the only remaining morphologic units of the cell.

The pancreatic cells occur in groups or islets that are regularly distributed throughout the organ. From one to three cells in an islet may be observed in cross-sections. Their distribution is most conveniently studied when the sections are stained for fat; the pancreatic cells do not contain fat and they stain strikingly in the counterstain of alum hematoxylin with the scarlet of the globules of fat as a differentiating, surrounding field (figs. 7 and 12). Once their general nature and situation in the acini have been determined, they may be observed well in tissue stained in hematoxylin and eosin.

The most striking pancreatic cells are those that are usually situated at the blind end of an acinus. This particular kind of cell is ellipsoidal, with the longitudinal axis at right angles to the basement membrane, one end of the cell being adjacent to the membrane; the other end, slightly attentuated and drawn out to form a duct, is in close contact with a crypt of the lumen. The membrane of the cell is distinct. The cytoplasm is deeply granular near the periphery. The nucleus is round to ellipsoid, possesses a distinct membrane, and contains one or more definite nucleoli (figs. 12 and 14). The cells measure 20 to 25 μ by 35 to 40 μ and the nuclei from 9 to 11 μ. The boundaries of other pancreatic cells are not as clearly defined as the boundaries of those just described, for, although this description of the cytoplasm and nucleus generally applies to the pancreatic cells, the merging of the islets and the hepatic cells lends to the former the appearance of delimitations (fig. 7). In some cross-sections of the acini the islets of pancreatic cells are thickly distributed and extend across one wall of the acinus, that is, from membrane to membrane, or are situated equidistant from the membranes. In some cases the ducts of two or three cells in an islet coalesce, and these common ducts empty into the same crypt of the lumen (fig. 14).

As there has been doubt of the presence of pancreatic tissue in what has been called the digestive glands of the crayfish, but which I call the hepatopancreas, it is essential that chemical tests substantiate cytologic studies. Accordingly, tests were made to determine whether or not the pancreatic enzymes, trypsin, amylase, and lipase, are present in the secretion of the organ. The hepatopancreases of twenty recently collected (June) animals were tested according to the method outlined by Hawk ('21). The three enzymes are present in the secretion.

### *The hepatic artery and the connective tissue*

The wall of the hepatic artery is composed of a layer of endothelial cells supported by an outer layer of fibro-elastic tissue; the latter consists of strands of cells, which are present throughout the interacinar spaces (figs. 3, 6, and 13). The blood vessels are associated throughout the liver with the fibro-elastic tissue.

The layer of endothelial cells is at most from 2 to 3 μ thick, appearing fiber-like, and the boundaries of the cells are indistinct. The cells are elongated and spindle-shaped, and the cytoplasm is finely drawn out from the nucleus toward the wall of the adjacent cell. One distinct, ellipsoid, reticulate nucleus regularly is present in each cell (fig. 13). The supporting wall, that is, the fibro-elastic layer, is thread-like and more lightly colored in hematoxylin and eosin than the inner layer of endothelial cells. The transverse walls of the cells are not regular, and they are distinguished with difficulty even under magnifications of 1500 or more diameters. The nuclei are ellipsoid or nearly round and larger than those of the endothelial cells, and usually contain a distinct nucleolus (fig. 15).

The connective tissue, which occupies the interacinar spaces, is laminar in connection with the transitional epithelium of the wall of the pyloric stomach. This connection is direct with the lateral walls of the stomach, and dense masses of the tissue pass across the dorsal surface of the stomach and among the acini of the hepatopancreas. Moreover, strands of connective tissue emerge ventrally from the transitional epithelium of the stomach, surround the hepatic ducts longitudinally, and infiltrate the interacinar spaces of the ventrolateral regions of the lobes of the organ (fig. 11).

The connective tissue is situated in the interacinar spaces along with two kinds of phagocytic cells (figs. 2 and 4). The latter are polyblasts. There is some doubt concerning the differentiation and the function of the so-called resting wandering cells, but I shall follow Maximow ('02) in designating these cells. The two kinds are clearly different.

In portions in the wall of the stomach the outlines of the cells of the fibro-elastic tissue are distinct. They are fusiform and the distinct nuclei conform to the shape of the cells.

The greatest diameter of the nuclei is from 8 to 10 μ and the least, from 4 to 6 μ. The nuclear chromatin is readily apparent and a large nucleolus usually is present.

The fibro-elastic tissue is not uniformly present in the interacinar spaces, but it surrounds, in some degree, each acinus; in some cases it invades the periphery of the acini, so that there is no sharp line of demarcation between it and the hepatic cells. As would be expected, the reticulum, of which the fibro-elastic tissue is the chief constituent, is more abundant near the hiluses than toward the periphery of the lobes.

The small polyblasts are usually situated in angles or crypts which result from the anastomosing of the fibers of the fibro-elastic tissue to which they apparently are attached by exceedingly minute fibrous threads (figs. 4 and 15). The cells are round or polyhedral, depending on the situation in which they are found. The nuclei are from 3 to 5 μ in diameter and occupy virtually the entire volume of the cells.

The large polyblasts are distinctly different in relation to the surrounding elements in appearance and form. They have no definite foci of occurrence, being found in, or adjacent to, the fibro-elastic tissue of the wall of the stomach and in the interacinar spaces (figs. 4 and 15). They are larger than either the cells of the fibro-elastic tissue or the small polyblasts, the diameter ranging from 12 to 15 μ. They usually are round, but occasionally an attenuation of the wall gives the appearance of an attachment to a fiber—a condition which is not constant. The nucleus usually conforms to the shape of the cell, but club-shaped nuclei are present and in some cases the nucleus is divided by amitosis. Demarcation between the nucleus and the cytoplasm is sharp; the nucleus stains a medium dark blue in hematoxylin and a wine-red in fuchsin, while the cytoplasm is neutrophilic. The cytoplasm apparently does not constantly stain in fuchsin, but appears refractive and granular. I have observed a completed division in which the volume of the cytoplasm of one resulting cell apparently was twice as great as that of the other cell and the quantity of the nuclear material in the one distinctly greater than in the other—a characteristic result of amitosis.

### THE BLOOD SUPPLY

The course of the blood from the heart to the acini of the hepato-pancreas may be demonstrated by the injection of India ink into the heart by means of capillary cannula. The hepatic artery bifurcates as it leaves the anteroventral surface of the heart, and the two branches pass slightly ventrad to the hiluses of the lobes. Within the lobes the bifurcations disperse into numerous smaller branches throughout the interacinar spaces.

The condition within the lobes may be determined by a study of serial cross-sections. In my description I shall refer to the supply in one lobe. About 200 μ exterior to the hilus the diameter of the artery is from 65 to 70 μ; at this point the first marked change occurs. A second bifurcation produces two primary branches, that is, those which immediately enter the lobe. One of these branches straightway enlarges to a diameter of about 120 μ, forming what appears to be a reservoir. This reservoir is preceded and followed by constrictions; the artery then passes into the nearest interacinar space. The second branch has three constrictions, but the two resulting reservoirs are about the diameter of the artery before it reaches the hilus. These two chief branches disperse secondarily throughout the interacinar spaces of the lobes, branching into capillaries from 2 to 4 μ in diameter (figs. 3, 6 and 9).

Although the capillaries are closely associated with the acini, the immediate supply of blood constituents to the cells is by osmosis. The blood vessels are supported by the fibro-elastic tissue, which is in contact with the basement membrane of the acini, and in some cases the supporting tissue infiltrates the acini on the periphery. I have demonstrated that, within twenty minutes after the injection of India ink into the heart, ink is not only in the basement membrane, but is in the peripheral cells, in some cases in the nuclei (figs. 5 and 10). There is no clear evidence of capillaries leading directly into cells, although, where there is fine infiltration of the acini by fibro-elastic tissue and consequently by capillaries, the cells are filled with India ink. This view is substantiated not only by examination of the hepatic and the pancreatic cells, which contained India ink, but by a careful examination of the ova. The latter lie in the oviducts dorsal to the hepatopancreas and the pyloric stomach; and, in a specimen in which the heart pumped India ink for twenty minutes before fixation, ink was observed in capillaries among the ova; ink was not observed to have entered the ova by capillaries.

## DEPOSITION OF FAT AND GLYCOGEN

Fat is stored in the hepatic cells. In the cells of specimens collected in September there is little evidence of its deposition; only a few obscure, minute globules are present. However, in specimens collected in June the globules of fat are large and abundant and they obscure practically all parts of the cells by their presence (fig. 7). It is necessary, therefore, to study the cells and their depositions of globules of fat solely on the basis of the arrangement of the latter.

The result of fixing the tissue in formalin and staining frozen sections in scarlet red and counterstaining in alum hematoxylin is that the outlines of the hepatic cells are clearly indicated by the arrangement of the (red) globules of fat. Indeed, in some cases the nucleus is splendidly shown by its reaction to the stain. In fully 50 per cent of the cells the membrane actually may be seen only under a magnification of 800 or more diameters. It is possible, but highly improbable, that the quantitative distribution of fat that I have indicated is due to artificial conditions: the liver was placed in formalin within thirty seconds after the animal had been removed from its natural habitat.

Glycogen is present in the hepatic cells of recently collected animals taken in September and June (fig. 17). However, the response to the differential stain, Best's carmine and alum hematoxylin, is not as pronounced as in the demonstration of the deposition of fat. The granules of glycogen are exceedingly minute (from 2 to 3 μ), whereas fat is deposited abundantly and in globular form.

It should be noted that the amounts of glycogen found in the hepatopancreas in September and June were about the same, while fat scarcely was detectable in September, but was abundant in June. Whether or not these facts are related to the feeding habits of the animal, I cannot say. There is food in the stomach during both of these seasons of the year and the animals were predaceous while they were in captivity without food.

## DISCUSSION

It is evident that the hepatopancreas of Cambarus is a tubular organ; a similar morphologic condition probably exists in members of the higher Crustacea, if not also in other highly specialized Arthropoda. My unpublished work on Melanoplus corroborates this view. The invertebrate hepatopancreas as represented by that of Cambarus is interesting in comparison with the liver of the vertebrates, especially the livers of Pisces, Amphibia, and Aves. Eberth ('67) has shown in his study on Cyprinus, Salamandra, and Gallus that the basis of structure of the livers is practically identical in that the lobules typically consist of a single row of cells, which delimits a lumen through which the bile ducts reach the lobules, and the blood capillaries lie in the interlobular spaces. The tubular condition, the single row of cells in the lobule, the bile ducts, and the blood capillaries of the livers of these vertebrates are present structure for structure in the hepatopancreas of Cambarus. However, a basement membrane encloses each acinus (lobule) in Cambarus and is continuous throughout the organ. Moreover, the anastomosing tubules in Cambarus are more conspicuous: the tubules (lumen) and the acini (lobules) are larger. It is doubtful whether the vascularity of the cells in Cambarus is as marked as in the vertebrates, in which one cell may be surrounded by as many as four or more capillaries. I have demonstrated that the capillaries enter the acini (lobules), but I have not demonstrated the exact condition therein. My observations point to the view that most of the blood supply is through the basement membrane and thence into the cells.

Shore and Jones ('89), in a study on the vertebrate liver, used some members of the groups employed by Eberth. Of the Pisces they used Petromyzon, Muraena, Pleuronectes, and Scyllium; of the Amphibia, Rana, Coecilia, and Triton; of the Reptilia, Testudo and Pelias; of the Aves, Gallus; and of the Mammalia, Felis, Talpa, Mus, and Sus. These authors conclude that the gland tissue of the liver in all types of vertebrates is an anastomosing network of tubules and that "there is no evidence of change of plan as we ascend the vertebrate scale." They further state that the mammalian liver is characterized by "a greater penetration of blood vessels between the secreting cells . . . . and a coincident growth of connective tissue along the portal branches so as to map out the organ into lobules. . . ."

The results of the present study seem to indicate that, while the structure of the hepatopancreas of Cambarus is on the same plan, it is more primitive than that of the vertebrate (especially mammalian) liver, and this primitiveness is indicated by the relatively small number of anastomosing tubules and their simplicity of arrangement and the extent of the distribution of the blood capillaries and the bile ducts. It is highly probable that the presence of the basement membrane also is a primitive condition.

## SUMMARY

The relation of the hepatopancreas of Cambarus virilis to the pyloric stomach, its histology and blood supply, and the deposition of glycogen and fat were studied. The organ consists of two lobes, united ventrally, which lie along the cardiac and pyloric stomachs and the intestine. The primary lobula-

tions are succeeded by secondary lobulations; the entire organ is a system of anastomosing tubules, the lumina of the acini. The tubules progressively converge to form a duct in each lobe which leads ventrolaterally into the pyloric stomach; the secretion of the gland is discharged through these two ducts into the pyloric stomach.

The acini consist of a single row of hepatic and pancreatic cells supported in part by a basement membrane, and the continuous interacinar spaces are the seat of fibro-elastic tissue and large and small phagocytic cells. The supporting tissue is laminar with that of the stomach. The arterial capillaries, composed of endothelial cells supported by fibro-elastic tissue, lie in the interacinar spaces. The blood supply, by osmosis, is by way of the basement membrane and thence into the cells.

The hepatic cells of specimens collected in September bear little fat; the cells of those collected in June are laden with fat. Glycogen likewise is deposited in the hepatic cells, and there is no difference between the quantity found in September and in June. The hepatic cells also probably contain deposits of biliverdin. The pancreatic cells bear little or no fat or glycogen. Chemical analysis indicates the presence of trypsin, amylase, and lipase.

The histologic structure of the hepatopancreas is homologous to that of the liver of Cyprinus, Salamandra, Gallus, and primitively similar and homologous to that of Felis, Mus, and Sus.

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## PLATE 1

### EXPLANATION OF FIGURES

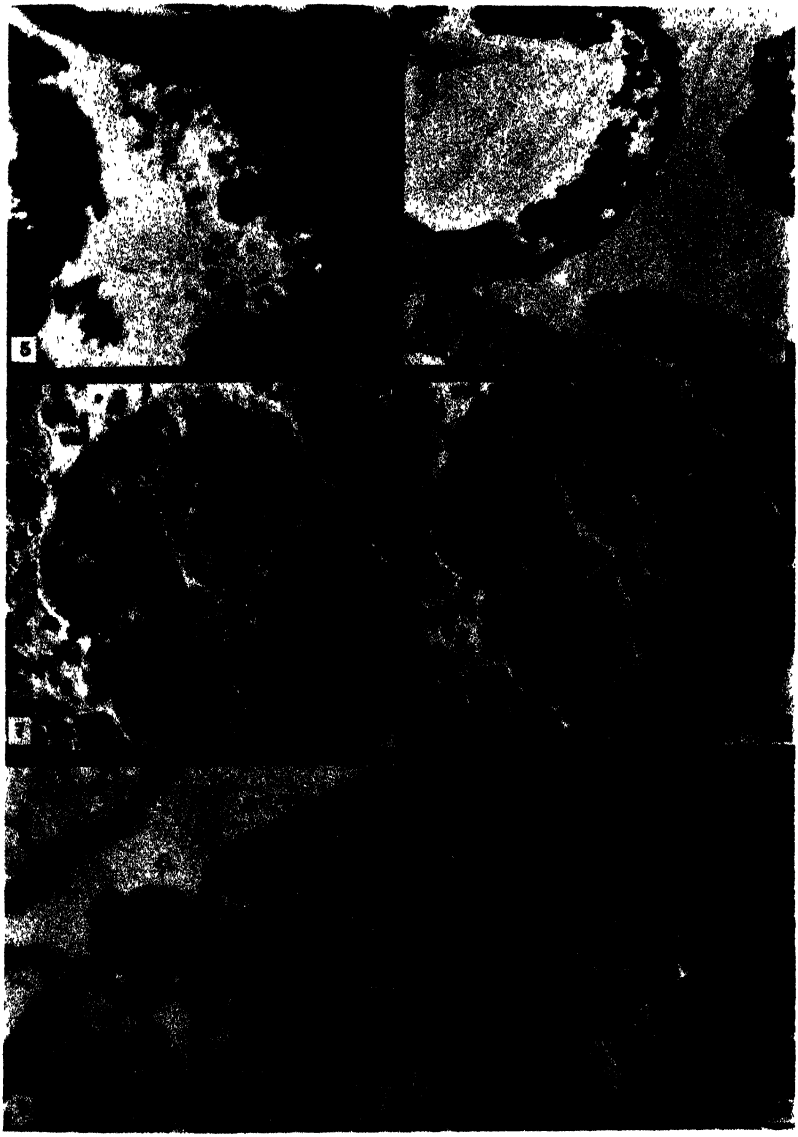
5 An injected arterial capillary: endothelial and fibro-elastic cells. × 350.

6 An injected arterial capillary: endothelial cells. × 600.

7 and 8 Cross-section of an acinus. The dark cells are pancreatic; the globules are fat in the hepatic cells. × 200.

9 Cross-sections of acini; arterial capillaries injected with India ink. × 600.

10 Cross-section of acini and an injected arterial capillary: India ink in the basement membrane. × 600.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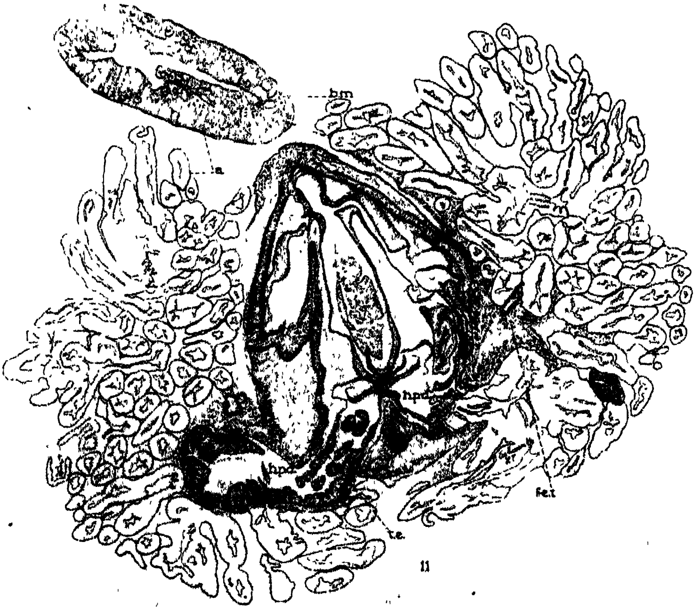
## PLATE 2

### EXPLANATION OF FIGURE

11 Cross-section of pyloric stomach and hepatopancreas in region of the ducts. × 100.



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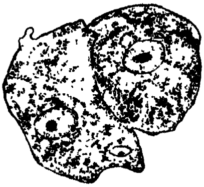
## PLATE 3

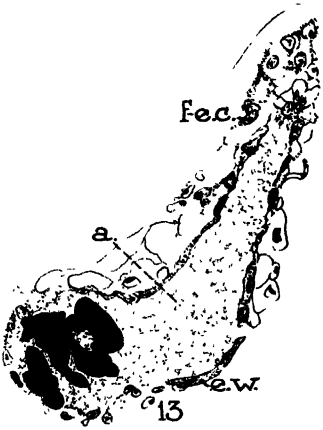
### EXPLANATION OF FIGURES

12 Pancreatic cells. × 450.

13 Longitudinal section of an injected arterial capillary: endothelial and fibro-elastic cells. × 450.

14 Cross-section of an acinus: pancreatic cells. × 450.





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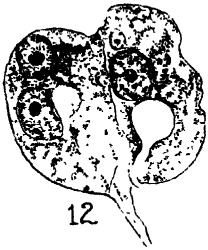

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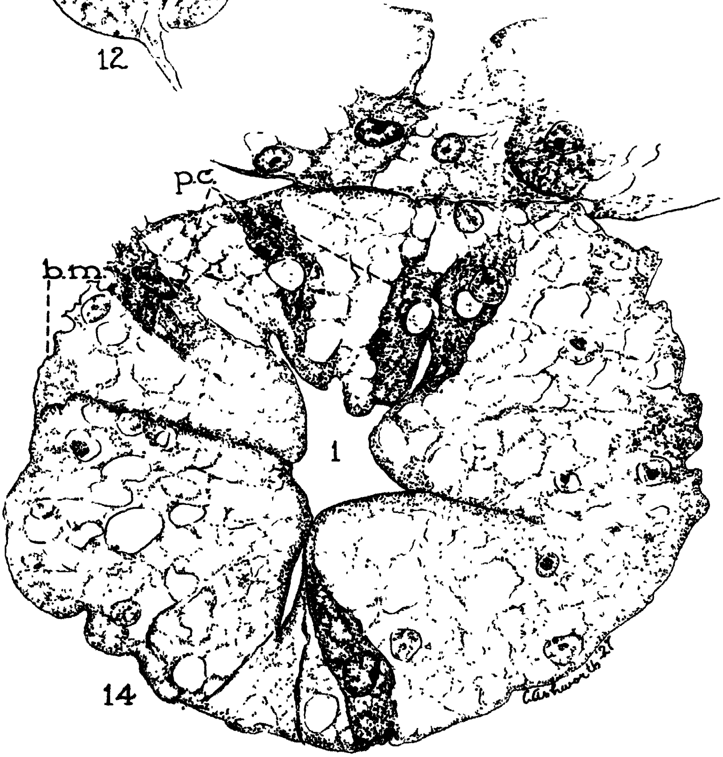

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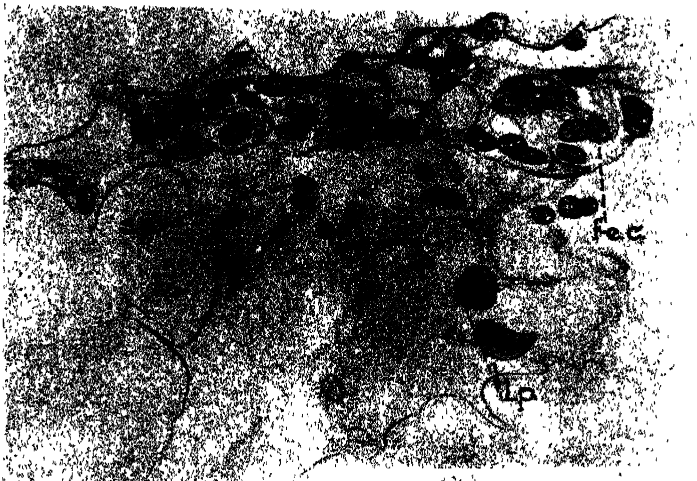
## PLATE 4

### EXPLANATION OF FIGURES

15 Fibro-elastic tissue and phagocytes from an interacinar space. × 450.
16 Full view of the hepatopancreas: *a*, dorsal; *b*, lateral; *c*, ventral.
17 Hepatic cells with deposits of glycogen. × 450.



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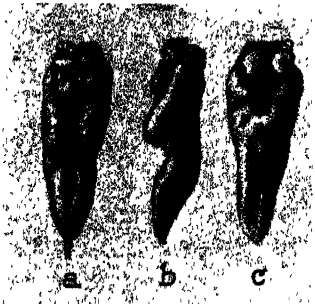

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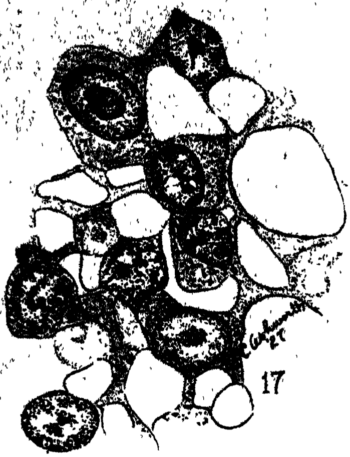

17

# THE MORPHOLOGY AND PHYSIOLOGY OF THE INVERTEBRATE LIVER AND HEPATOPANCREAS[1]

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NINE FIGURES

AUTHOR'S ABSTRACT

Modifications of the gastro-intestinal tract of Nereis virens are two dorsolateral esophageal cecums, directed anteriorly and posteriorly from the point of attachment. The cecal and esophageal lumina are continuous. Each cecum is composed of acini, possessing a row of elongated cells, basement membrane, and intima. Fibro-elastic tissue is present. The elongated cells contain glycogen and fat.

The hepatopancreas of Asterias vulgaris consists of two lobes in each ray, and the numerous acini empty into the bifurcated hepatopancreatic duct which leads into the pyloric stomach. The hepatic cells are columnar and contain glycogen and fat. Pancreatic cells lie in the midregion of the acini.

The lobulate liver of Loligo pealii lies on the ventral surface of the duodenum. A capsular membrane envelops the organ. The acini possess basement membrane, columnar cells, and intima, their lumina anastomose, forming a common duct, which leads into the blind sac. The hepatic cells contain fat.

The hepatopancreas of Melanoplus femur-rubrum consists of six cecums histologically continuous with the digestive epithelium of the pyloric stomach. Each cecum consists of simple and compound acini which empty into a duct leading to the pyloric stomach. Each acinus possesses basement membrane, columnar hepatic cells, pancreatic cells, and intima. The pancreatic cells occur in islets, usually adjacent to the basement membrane. The hepatic cells contain glycogen and fat.

## INTRODUCTION

In a previous paper[2] I presented the results of a study on the hepatopancreas of the common crayfish, Cambarus virilis, and I indicated that a similar study would be made on Nereis virens, Asterias vulgaris, Loligo pealii, Melanoplus femur-rubrum, and Balanoglossus aurantiacus. It was found that Balanoglossus aurantiacus is unsuitable for this study unless special care can be taken in the preparation of material. As I have not had the opportunity of making my own collections, I have had to forego this study. I have, therefore, studied the esophageal cecums (diverticula) of Nereis virens, the hepatopancreas of Asterias vulgaris, the liver of Loligo pealii, and the hepatopancreas of Melanoplus femur-rubrum, with particular reference to the histologic structure, secretion of pancreatic enzymes, and the deposition of fat and glycogen in the hepatic cells.

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[1] From the Division of Experimental Surgery and Pathology.

[2] Dorman, H. P., 1928. The morphology and physiology of the hepatopancreas of the Cambarus virilis. This journal, pp. 505–536.

It should be emphasized that, although I was able to demonstrate that the organ of Cambarus virilis, which has been called hepatopancreas or liver by various authors, is in reality hepatopancreas, the results of the study on Nereis virens are not as sharply defined. The differences that obviously may be surmised after gross examination of the esophageal cecum of Nereis are more strongly emphasized in the results of microscopic study, and I shall point out these differences.

As I have reviewed the literature on the liver and the hepatopancreas of the invertebrates with reference to histologic structure and the deposition of fat and glycogen (loc. cit.), I shall not review the work of others here.

## MATERIALS AND METHODS

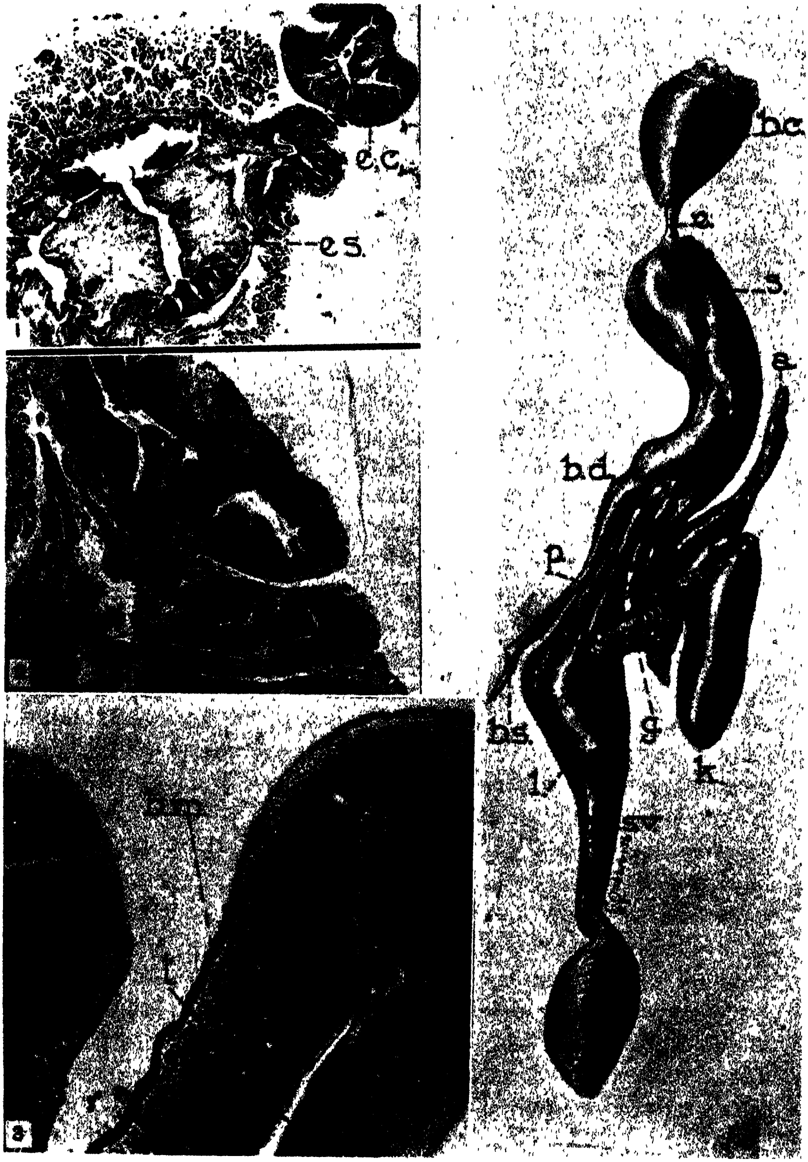
Specimens of Nereis virens and Asterias vulgaris were collected and fixed while fresh by members of the staff of the Marine Biological Laboratory, Woods Hole. Nereis virens was collected in October and Asterias vulgaris and Loligo pealii in May. Specimens of Nereis virens simply were dropped into killing fluids on collection, but the hepatopancreas of Asterias vulgaris was removed from the animals before fixation. The material used for the determination of the presence of enzymes was finely ground in a mortar, covered with 30 per cent alcohol, and shipped to the laboratory in Rochester, Minnesota. The tests of the specimens of Loligo pealii were slit before the entire animal was fixed, so that the fluid would readily reach the liver. Adults and nymphs of Melanoplus femur-rubrum were collected at Rochester in September and June, respectively.

The material was prepared for examination by three chief methods: 1) for general study, the tissue was fixed in Zenker's fluid, sectioned serially at 10 μ, and stained in methylene blue and eosin, in Delafield's hematoxylin and eosin, and in Mallory's triple connective-tissue stain; 2) for the demonstration of the deposition of fat, the organ was fixed for from twenty-four hours to several days in 10 per cent formalin, and frozen sections of 10 to 15 μ were stained in scarlet red and counterstained in alum hematoxylin; 3) for the demonstration of the deposition of glycogen, the organ was fixed for from twenty-four to seventy-two hours in absolute alcohol, and celloidin sections of 10 μ were stained in Best's carmine and counterstained in alum hematoxylin.

### *Esophageal cecums of Nereis virens*

The only modifications of the gastro-intestinal tract in Nereis virens that may be interpreted as accessory organs of digestion, such as hepatopancreas or liver, are two esophageal cecums situated dorsolaterally. These cecums arise from the anterior portion of the muscular esophagus and are directed anteriorly and posteriorly parallel to the esophagus. The two points of evagination from the esophagus are not always at the same level; they frequently occur at levels as much as 0.15 mm. apart. Then, too, the distances from the duct to the anterior and posterior ends of the cecum are usually in the ratio of 5: 17. In a specimen from 13 to 14 mm. long the cecum is 2.3 mm. long, and the greatest amplitude in cross-section is 0.13 by 0.05 mm.

The lumen of each cecum is continuous with that of the esophagus, and so with the entire gastro-intestinal tract. In fact, the cross-sections of the cecum closely resemble the cross-section of the esophagus (fig. 1). The chief superficial difference lies in the fact that the epithelium of the cecum is in folds or convolutions (figs. 1 and 2); whereas that of the esophagus is relatively simple. Viewed as a whole, the lumen of the esophagus is a dorsoventral slit bounded by continuous acini; and, while each cecum clearly has such a slit from which extend compound acini, it is more variable in its relation to the whole. The lumen is formed into a duct which leads into the esophagus, and there is no noticeable change



Figures 1 to 4

in the character of the tissues as the transition from cecum to esophagus occurs (fig. 1). There is more musculature in the walls of the esophagus than in the cecum, but the epithelial tissues are at least superficially the same. This suggests several functional possibilities: 1) the cecum merely serves as an enlargement of the digestive epithelium; 2) the cecum as well as the esophagus is glandular, and its highly acinous structure merely permits the formation of a greater quantity of secretion, no digestion actually taking place in the cecum; 3) digestion occurs largely in the esophagus, and some absorption at least occurs in the cecum. The size of the duct as it progresses from cecum to the esophagus probably is not sufficiently large to accommodate the passage of food into the cecum for digestion. The usual diameter of the duct is from 5 to 8 μ. I am inclined to believe that the second hypothesis is more likely correct than either of the other two.

The outer surface of the cecum is covered by a thin sheath of transversely arranged fibro-elastic tissue; the cells are fusiform with prominent nuclei which stain brilliantly in alum hematoxylin. The nuclei measure up to 5 μ. The sheath varies in thickness from one to three cells. The area which lies directly beneath the sheath and between the sheath and the epithelium of the acini is predominantly muscular and

**Fig. 1** Cross-section of the esophagus and one of the esophageal cecums of Nereis virens, showing transition from the lumen of the cecum to the lumen of the esophagus. *e.c.*, esophageal cecum; *es*, esophagus. 10 μ, × 30.

**Fig. 2** Cross-section of a portion of one of the esophageal cecums of Nereis virens, showing the acini. 10 μ, × 60.

**Fig. 3** Cross-section through the acini of the hepatopancreas of Asterias vulgaris, showing (light area) the hepatic cells and the pancreatic cells (inner part of the acinus). *b.m.*, basement membrane; *l*, lumen. 10 μ, × 120.

**Fig. 4** The gastro-intestinal tract of Loligo pealii, ventral view. *a*, anus; *b.c.*, buccal cavity; *b.d.*, bile (hepatic) duct; *b.s.*, blind sac; *e*, esophagus; *g*, gill; *k*, kidney; *l*, liver; *p*, pancreas; *s*, stomach; *s.v.*, spiral valve. Natural size.

ABBREVIATIONS

*b m*, basement membrane
*f c*, fibroserous coat
*h c*, hepatic cells
*i*, intima
*l*, lumen
*p c*, pancreatic cells

infiltrated by the connective tissue, which extends into the epithelium. The muscular arrangement is variable; but, in general, the fibers are radial; in certain situations the fibers are tangential to the sheath and gradually curve inward until the axes of the inner halves are at right angles to the sheath. These fibers extend to the epithelium; in other situations a layer of transversely striated muscle lies from 5 to 25 μ beneath the sheath and the intervening space is filled with muscle fibers. In short, there are gaps in the transverse layer of muscle fibers, and in these situations the muscle fibers extend inward from the fibro-elastic sheath to the epithelium.

The glandular epithelium, which is separated from the lumen by the intima, is composed of a single row of elongated cells (fig. 2). These cells are about 22 by 8 μ and bear one or two darkly staining nuclei. The cell membranes are indistinct and the cytoplasm contains basophilic granules. It may be said that these cells have every appearance of being hepatic cells. They are similar to the hepatic cells which I have described for Cambarus virilis. Glycogen and fat-granules are deposited in the cells of specimens that were collected in September, as, indeed, they are also deposited in the epithelial cells of the esophagus.

### *The hepatopancreas of Asterias vulgaris*

The hepatopancreas of the starfish has been described many times as the pyloric or hepatic cecums, and various descriptions of these organs are given in text-books of zoölogy. In my study of the literature I have not found any specific evidence that the cecums constitute a liver or pancreas or a conjunction of the two organs. It is commonly known and asserted by students of marine animals that the cecums are a digestive gland, but I believe that there has been thus far no demonstration of their specific function. I have referred (loc. cit.) to Stone's ('97) effort and failure to ascribe to the cecums the function of glycogen deposition.

Since the general structure of the cecum is well known, I shall devote little attention thereto and consider the microscopic structure, the deposition of fat and glycogen, and the production of pancreatic enzymes (amylase, lipase, and trypsin) as indications that the functions of the liver and pancreas are simulated in the starfish. Because of the puzzling appearance of the tissue and my inability on first examination to differentiate clearly the hepatic and pancreatic cells, the determinations for enzymes were made first. The enzymes mentioned were found in the secretion of the cecums of freshly collected specimens, and this and other evidence, which I shall present, justify designating the cecum as hepatopancreas.

I have used only the hepatopancreas of the five-rayed starfish, Asterias vulgaris, in which two of the ten lobes of the organ occupy virtually the entire cavity in each ray. Macroscopically it may be determined that the hepatopancreatic duct, which leads from the two lobes in each ray into the pyloric stomach and bifurcates about the point it passes from beneath the aboral disc. The bifurcations are identical and serve identical lobes of the organ. In fact, the lobes in all five rays are identical. Viewed aborally, then, the bifurcations are seen first, and the profusely branched cecum, leading at frequent intervals into the bifurcations, is oral; the arrangement of the cecum is such that each bifurcation, viewed aborally, is median to the lobe it serves. The lobes are yellow and spongy, and the anastomosing, tubular arrangement is suggestive of the hepatopancreas of Cambarus.

The acini are extremely convoluted and there is no definite criterion to determine the limits of an acinus. The outer and the inner surfaces, that is the protected and the secreting areas, are distinguishable. However, the former is supported by connective tissue and a basement membrane, and the latter consists of a mucous membrane or intima (fig. 3). This connective tissue envelops the ducts and the lobes. In cross-section the walls of the acini usually extend laterally, the closed ends being at the lateral limits of the lobe. The acini anastomose freely, interacinar spaces are present, and the lumina are continuous one with the other. Thus, the plan of structure of this organ in Asterias is similar to that of Cambarus.

The lobes measure up to 7 mm. dorsoventrally and 3 mm. from side to side. The acini are not longer than 3 mm., and the walls are from 0.2 to 0.3 mm. thick.

The hepatic cells lie in two or three rows, the outer of which is in contact with the basement membrane, while the pancreatic cells occupy the inner part of the wall of the acinus. The hepatic cells are of the columnar type with elongated nuclei, usually one to the cell. A large number of interstitial cells are present, some of which are squamous and some of which are spindle-shaped. The measurements vary from 10 to 20 μ in length. The nuclei are round to ellipsoid and are exceedingly small.

The pancreatic cells are more elongated than the hepatic cells, some of them being as long as 30 μ. In the midregion of the wall of the acinus they are compact and closely grouped and the nuclei stain intensely in alum hematoxylin, whereas near the lumina they are loosely associated and appear to be fibrous. The cells in the midregion are exceedingly granular, and in this respect resemble the pancreatic cells.

I have already referred to the secretion of amylase, lipase, and trypsin as evidence that the organ performs a pancreatic function. The hepatic function is indicated by the deposition of fat and glycogen in the hepatic cells. The hepatopancreases of specimens collected in May were removed in fresh condition and immediately fixed in formalin and absolute alcohol. Frozen sections of the formalin-fixed material were stained in scarlet red and counterstained in alum hematoxylin, while celloidin sections of the alcohol-fixed material were stained in Best's carmine and counterstained in alum hematoxylin. The sections stained in scarlet red and alum hematoxylin disclosed a remarkable condition: a line of demarcation between the hepatic cells, in which large and small scarlet-stained globules of fat were deposited, and the pancreatic cells, which were stained by the alum hematoxylin (fig. 3). As in Cambarus, the delineations of the hepatic cells were clearly indicated by the arrangement of the globules of fat. The deposition of glycogen, the granules of which were stained by Best's carmine, was intensely shown in the hepatic cells, and there was some evidence of glycogen in the pancreatic cells.

### *The liver of Loligo pealii*

The liver of the squid, Loligo pealii, is a lobulate organ which lies next to the heart along the ventral surface of the duodenum, and is attached to the gastro-intestinal tract by a heavy membrane (fig. 4). A sagittal fissure indicates that the organ in its present adult condition probably is the result of the fusion of two unequal lobes, which, fused as they are, give off secretory capillaries into a common hepatic duct. The duct empties into the midportion of the blind sac. I have examined three specimens, and the average length of the livers was 2.1 cm.

Microscopic examination discloses that the structure of the liver is not unlike that of the hepatopancreas of Cambarus virilis. In fact, the two organs are strikingly similar in general, but some of the details in Cambarus are lacking in Loligo. The liver is enveloped by a capsular membrane that is continuous with the connective tissue throughout the interacinar spaces; the membrane is present in Cambarus, but I did not detect its relation to the connective tissue of the interacinar spaces. However, the secondary lobulations which delimit anastomosing tubules, or the acini with the anastomosing and converging lumina, as well as the interacinar spaces in which the blood capillaries and the fibrous connective tissue are situated, are common to both species.

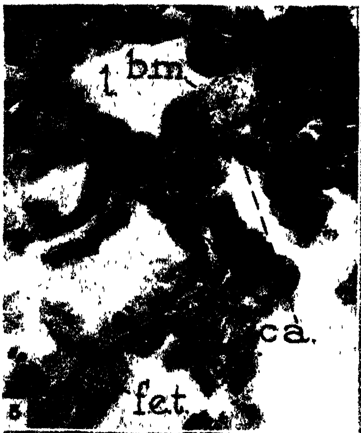
The size of the acini varies considerably, and, due to the simple and compound arrangement of the entire organ, it is not feasible to determine exact dimensions. In brief, it is impossible to ascertain in every case just where a simple or a compound acinus begins and ends. However, an average acinus in cross-section is from 70 to 80 μ by 40 to 45 μ and the lumen or tubule 2 to 10 μ broad, depending on the form of the unit. A basement membrane surrounds each acinus and is continuous with the membranes of other acini, as well as with the capsular membrane of the entire organ (fig. 5).

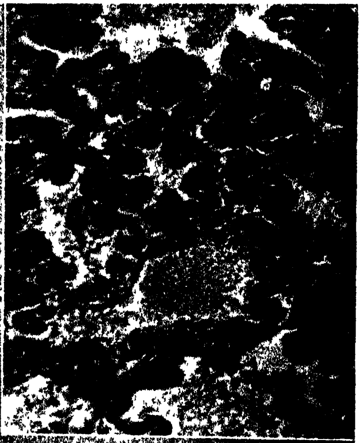
A single row of hepatic cells extends from the basement membrane to the intima, the latter separating the cells from the lumen. In general, the cells are of the columnar type, but spindle-shaped (interstitial) and squamous cells also are present (figs. 5 and 6). The latter kinds usually are situated in curvatures of the wall and the broad ends of the squamous cells are toward the convex surface. Naturally, there are extreme variations among the sizes of the cells, which range from 6 to 9 μ by 18 to 25 μ, and they are nearly always uninucleate. The nuclei are round to ellipsoid and measure from 5 to 12 μ.

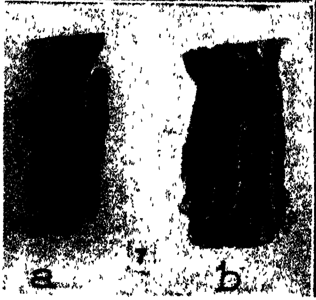
The lumina of the acini, or the tubules, which anastomose and converge to form the hepatic duct, serve as 'bile' capillaries. There is evidence in cross-section that the secretion of the organ contains a greenish-yellow pigment, but I have not had fresh material on which to verify this assumption.

I cannot estimate the exact gross blood supply of the organ, but sections show the branching arterial capillaries, which ramify throughout the interacinar spaces (fig. 5). This evidence emphasizes the fact that the relation of the arterial capillaries to the hepatic cells is similar to that in the hepatic and pancreatic cells in Cambarus. The capillary wall consists of a layer of endothelial cells, which is supported by fibrous tissue. The capillaries lie along and in contact with the basement membrane, and it appears that the supply of blood to the cells is by osmosis.

The specimens that I have used were collected in May. In tests for fat, fixation of tissue in 10 per cent formalin was followed by staining in scarlet red and counterstaining in alum hematoxylin. Fat-globules were shown in the cells (fig. 5). Cytologic tests for glycogen were not satisfactory, probably on account of imperfect fixation in absolute alcohol.







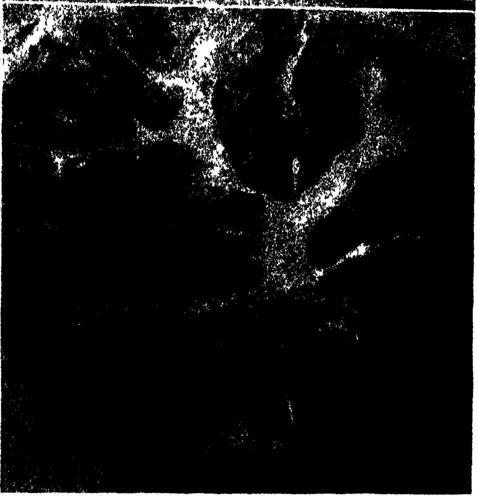


**Fig. 5** Cross-section of the liver of Loligo pealii: two complete and parts of other acini. *b.m.*, basement membrane; *ca*, capillary; *f.e.t.*, fibro-elastic tissue; *l*, lumen..

**Fig. 6** Section of the liver of Loligo pealii, showing the acini, the interacinar spaces, and two arterial capillaries. 10 μ, × 120.

**Fig. 7** The pyloric cecums (hepatopancreas) and the cardiac and pyloric stomachs of Melanoplus femur-rubrum. *a*, semidiagrammatic side view of a cecum; *b*, the dorsal surface of the cardiac and pyloric stomachs. × 5.

**Fig. 8** Cross-section of one cecum of the hepatopancreas of a nymph of Melanoplus femur-rubrum, showing fat in the hepatic cells. 10 μ, × 120.

### *The hepatopancreas of Melanoplus femur-rubrum*

The hepatopancreas of the locust, Melanoplus femur-rubrum, consists of six cecums which are evaginations of the anterior portion of the true stomach (chyle stomach or ventriculus) (fig. 9, A). The cecums lie parallel to the stomach and extend over a part of the esophagus (fig. 7). The points of attachment, which, histologically, are one, are at the same level, and these points are symmetrically arranged with reference to the circumference of the stomach. Usually about four-fifths of each cecum is anterior to the points of attachment, but in some cases these lengths are reduced to one-third. There are variations among the lengths and diameters of the cecums of a first-year specimen; the lengths vary from 7 to 7.5 mm. and the diameters from 0.7 to 1.9 mm.

On gross dissection of the animal, the cecums are relatively obscure. Apparently, they are secured to the ventriculus by the malpigian tubules that empty into the anterior of the intestine. The relationship that exists between the hepatopancreas and the excretory organs I shall discuss further on. On account of the minuteness of the organs, the morphologic relation of the cecums to the ventriculus can be determined only by microscopic study.

The acinus is the structural unit of the hepatopancreas. Each cecum is composed of simple and compound acini, the lumina of which converge into a common lumen or duct (fig. 9, A and B). This duct is continuous with the lumen of the ventriculus. The acini are, therefore, radially arranged, and they present such an appearance even in the compound condition. A fibroserous coat envelops the cecum and is continuous at the points of attachment to the ventriculus.

The acinus, with its closed end adjacent to the fibroserous coat, is composed of these elements: hepatic cells, pancreatic cells, basement membrane, intima, and lumen of the tubule (fig. 9, B). The dimensions of the acini in an adult specimen collected in September vary widely. The lengths range from 0.15 to 0.65 mm. and the diameters, from 0.12 to 0.18 mm. The diameters of the lumina vary from 0.15 to 0.35 mm.

The hepatic cells are of the columnar epithelial type, with the long axes at right angles to the basement membrane and the intima. Uusually, one cell extends from membrane to membrane or from the interacinar space to the lumen. A small percentage of the cells are of the interstitial type, these cells being so arranged among the columnar cells that their attenuations lie adjacent to the basement membrane and to the intima. At points of curvature in the acini the cells invariably are squamous with the broad end of the cell adjacent to the convex surface. The dimensions of these cells are from 50 to 65 μ by 14 to 20 μ. The cell membranes are indistinct and the cytoplasm is non-granular and consistent except that a vacuole usually lies between the single nucleus and the intima. The round to ellipsoid nuclei stain brilliantly in methylene blue, and the membranes and the chromatin granules are distinct. Nucleoli regularly are present, one to each nucleus. The round nuclei range in diameter up to 13 μ; the ellipsoid nuclei measure 12 μ by 18 μ.

The pancreatic cells occur in groups or islets, as in vertebrates, which are distributed in the acini throughout the hepatopancreas (fig. 9, B). The islets extend from the basement membrane to the intima, but more than half of the cells in a group are arranged next to the interacinar space. Squamous cells, with the large ends adjacent to the smaller, spindle-shaped or round cells, extend their attenuated ends toward the intima and reach to it. As a rule, there are at least three and not more than eight islets of pancreatic cells in any one acinus. From six to fifteen cells compose an islet.

The smaller pancreatic cells are little more than intensely staining nuclei, for the cytoplasm and the cell membrane are relatively insignificant. The nuclei are round to ellipsoid and their greatest diameter is 13 μ. The cytoplasm is granular and vacuoles are absent. The larger squamous cells, which probably serve as pathways to the tubules for the secretion of the smaller, more remote cells are similar to the squamous hepatic cells in size and in the presence of the vacuoles. However, the cytoplasm of these squamous, pancreatic cells is granular, and that of the hepatic cells is not.

In the region of attachment of the cecum to the stomach the digestive tract is lobulate in cross-section. That is, the fusion of the ventriculus and the six cecums present the appearance of a single canal with six peripheral lobes, all having a common lumen. In the midregion of attachment the ventricular folds of digestive epithelium are replaced by the cells of the acini, but the acini are not as accentuated as they are in the more distal regions. They simply are invaginations of a wall of hepatic and pancreatic cells, which replace the digestive epithelium.

The union of the cecum at the points of attachment to the ventriculus indicates not only the unity of the hepatopancreas, but also its structural part in the digestive tract. Its functional rôle in digestion also is clear. Food in the process of digestion is found throughout the ventriculus and in the cecum, at least from the points of attachment to midway toward the posterior apexes of the cecum.

Associated with the fibroserous coat of the cecum are layers of reticular tissue. Usually one or two strands are present on each of the inner and outer surfaces. The reticulum of the inner surface is therefore associated with the basement membrane of the acini. Indeed, the interacinar spaces are the chief foci of the reticular tissue, and the fibers are closely related to the hepatic and pancreatic cells near the lower ends of the acini. However, some acini are more infiltrated than others with this tissue, and it appears in the lumen of the cecum, being associated with the nephridiums of the malpighian tubules.

The malpighian tubules, that are abundantly distributed in the peritoneal cavity, are attached to and closely associated with the fibroserous coat. These nephridiums are irregularly situated on the outer surface, and they enter one (in some cases, two) of the six cecums by way of a crypt, occupying, together with the reticular tissue, virtually all of the lumen.

The hepatopancreas of specimens collected in September show little evidence of depositions of fat and glycogen in the hepatic cells. The globules of fat and the granules of glycogen in the hepatic cells are rare and the latter are indistinct. However, in nymphs collected in June, fat and glycogen are abundantly distributed in the cytoplasm of the hepatic cells. Fat depositions, demonstrated by scarlet red and differentiated by alum hematoxylin, are minutely globular, and the delineations of cells are even more clearly indicated than by a general stain (fig. 8). In some cells the globules form a smooth plane of cytoplasm; in others, the outlines

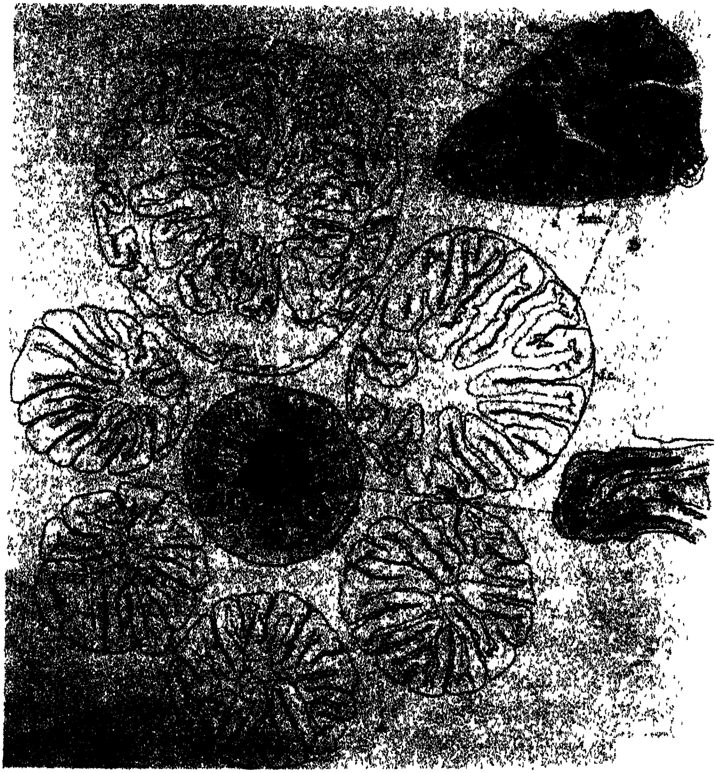


**Fig. 9, A** Cross-section of the pyloric stomach and the pyloric cecums (hepatopancreas) of Melanoplus femur-rubrum. 10 µ, × 50.

**Fig. 9, B** Detailed drawing of an acinus. × 250.

**Fig. 9, C** Detailed drawing of a fold of the digestive epithelium of the pyloric stomach. × 250.

of the globules delimit clear areas, and this reaction indicates that fat is not deposited homogeneously even during the period of the year that it is more abundant in the hepatic tissue. The clear areas simply denote parts of the cytoplasm free from fat deposition. The nuclei are stained remarkably well in the alum hematoxylin and form, with the scarlet red of the fat-globules, a beautiful histologic picture.

As in the hepatopancreas of Cambarus virilis, the pancreatic cells are stained only by the alum hematoxylin; clearly, the differential in function of the hepatic and pancreatic cells is shown by their responses to stain. The outlines of the islets of pancreatic cells and their situations in the acini are easily determined, for, if the hepatic cells are considered the background of the organ, the islets of pancreatic cells stand out in relief (fig. 8).

## DISCUSSION

Although the results of the histologic study of the esophageal cecums of Nereis virens suggested that these structures probably constitute a primitive hepatopancreas, the evidence derived was not sufficient to warrant such a conclusion. In order to determine the morphologic entity and physiologic significance, one should have access to fresh material. However, by the use of material prepared by professional collectors, I was able to demonstrate a marked similarity of the structure of these cecums to the structure of the pyloric cecums of Melanoplus femur-rubrum, which constitute in the latter the hepatopancreas. The most striking point of distinction lies in the fact that a cecum of Nereis possesses more musculature than one of the six pyloric cecums of the hepatopancreas of Melanoplus. In Nereis, as in Melanoplus, there is evidence of an acinous organ, each acinus possessing a basement membrane which supports a single row of cells. However, I have no evidence that these cells are of two kinds, hepatic and pancreatic.

A comparison of the hepatopancreas of Asterias and Melanoplus with that of Cambarus (loc. cit.) emphasizes that the organs are comparable in every histologic detail. Indeed, the liver of Loligo logically may be included in this comparison if it is considered that the liver and pancreas in the latter are entities. The liver is a tubular organ of anastomosing acini. The acini each possess a basement membrane, a single row of cells, and a lumen which serves as a bile capillary. The arterial capillaries are situated in the interacinar spaces, and fibro-elastic tissue is present in these spaces. Fibro-elastic tissue likewise is present in these spaces of the hepatopancreas of Melanoplus, but its distribution in the hepatopancreas of Asterias does not seem to be similar.

The evidence seems to show, therefore, that the hepatopancreas of Cambarus (loc. cit.), Asterias, and Melanoplus, the liver of Loligo, and the esophageal cecum of Nereis are similar in structure, and that the hepatopancreases in the three animals are similar in function.

## SUMMARY

Modifications of the gastro-intestinal tract of Nereis virens, which probably are homologous with the hepatopancreas of Cambarus, are two dorsolateral esophageal cecums. These are directed anteriorly and posteriorly from the point of attachment and parallel to the esophagus. The lumen of each cecum is continuous with the lumen of the esophagus. Each cecum is composed of acini, each of which possesses a single row of elongated cells bounded on the periphery by the basement membrane and separated from the lumen by the intima. Fibro-elastic tissue envelops each cecum and infiltrates the acini. Glycogen and fat are deposited in the elongated cells of specimens collected in September. It is probable that esophageal cecums secrete digestive fluid.

The hepatopancreas of Asterias vulgaris consists of two lobes in each of the five rays which lead into the pyloric stomach by means of the hepatopancreatic duct; the duct bifurcates as it passes from under the aboral disc. The lobes are identical and profusely branched into acini, each of which possesses a basement membrane and an intima. The lumina of the acini anastomose and finally lead into the hepatopancreatic duct. The hepatic cells lie in two or three rows, the outer of which adjoins the basement membrane. These cells are columnar and in the cells of specimens collected in May fat and glycogen are deposited. The pancreatic cells lie in the midregion of the acini. They secrete amylase, lipase, and trypsin.

The liver of Loligo pealii is a lobulate organ, which lies on the ventral surface of the duodenum. The organ is enveloped by a capsular membrane, which is continuous with the connective tissue throughout the interacinar spaces. The acini, the lumina of which anastomose and form a common hepatic duct which leads into the blind sac, possess a basement membrane, a single row of columnar cells, and an intima. The blood capillaries lie in the interacinar spaces. Fat is deposited in the hepatic cells of specimens collected in May.

The hepatopancreas of Melanoplus femur-rubrum consists of six pyloric cecums, which are histologically continuous with the digestive epithelium of the pyloric stomach. A fibroserous coat envelops each cecum. Each cecum is composed of simple and compound acini the lumen of which converge into a common duct which, in turn, leads into the pyloric stomach. The acini each are composed of a basement membrane, hepatic and pancreatic cells, the intima, and the lumen of the tubule. The hepatic cells are columnar and a single row lies between the basement membrane and the intima. The pancreatic cells occur in islets of three to eight cells, which may likewise extend from the basement membrane to the intima, but more often lie adjacent to the basement membrane. The reticular tissue, which is associated with the fibroserous coat, lies in the interacinar spaces. Fat and glycogen are abundantly deposited in the hepatic cells of specimens collected in June.

# THE METABOLISM OF AMINO-ACIDS BY PARAMECIUM CAUDATUM[1]

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THREE FIGURES

The object of the present study is to determine by quantitative means the rate at which a given number of paramecia can utilize the various amino-acids and to study factors influencing this rate.

The method consisted in testing the rate of utilization of isolated and mixtures of amino-acids by a known number of Paramecium caudatum. The amino-acids were used in 0.1 per cent solution and were tested, by the Henriques-Sorensen formol titration method for amino-acids, at the beginning and after the paramecia had lived in these amino-acids for twelve hours. The difference between these two tests, figured in percentage, is considered as the amount utilized by the paramecia. The part played by bacteria was found to be negligible when isolated amino-acids were studied. The influence of temperature on the rate of utilization of the amino-acids has been studied, and the results show a direct relationship between the two.

The rate of utilization was decreased by anaesthetics and nitroglycerin. A careful study has been made of a number of isolated amino-acids and the rate of utilization of each discussed in relationship to the others studied. An explanation of why one amino-acid was used more than another amino-acid was sought for, but not found. A detailed discussion of the above points has been presented in the body of the paper

That protozoa can metabolize various protein substances, such as bacteria, egg albumen, and other nitrogenous fragments, has long been known. The early investigators in this field have shown that many unicellular organisms have the necessary enzymes to enable them to digest animal proteins and utilize the end products for maintenance and growth. A few workers have presented evidence showing that the nitrogen metabolism of lower organisms differs from that in higher animals in that some protozoa do not require the essential amino-acids for growth, but may obtain their nitrogen from simpler sources of an inorganic nature. The object of the present study is to determine by quantitative means the rate at which a given number of paramecia can utilize the various amino-acids and to study some factors influencing this rate.

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[1] Data taken from the thesis submitted to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the Ph.D. degree.

## EXPERIMENTAL METHODS USED IN THIS STUDY

The protozoa used throughout this study were pure cultures of Paramecium caudatum, which were grown in large numbers in glass jars of about two gallons' capacity. The culture medium was made from boiled lake water by adding dried, lake-lily leaves which had been dampened and heated in a hot-air oven to 90°C. for one-half hour just before using, in order to destroy any protozoan spores that may have been on the leaves. Since the concentration of this medium can vary within wide limits, it is not necessary to measure the water or weigh the leaves in order to get abundant growth. By this method a pure culture of paramecia has been kept in the laboratory for over three months without any sign of depression, provided about one pint of boiled lake water and a very small handful of the lake-lily leaves are added daily. The entire contents of each jar was emptied about every three weeks, a fresh medium was prepared, and paramecia added from neighboring jars. One quart of infusion is a sufficient amount on which to start a culture and also leaves ample room in the jar for the addition of the daily food and water.

The paramecia grew in great numbers in cultures of this kind and collected as white masses at the top of the medium. When a quantity of paramecia was wanted, these white masses were skimmed off and strained through cheesecloth in order to remove any bits of leaves that might be present. The strained liquid was then centrifuged at sufficient speed to throw the paramecia to the bottom, the supernatant liquid and dirt were aspirated off with a suction flask and the organisms washed out of the centrifuge tube with a little water. By this means 5 to 15 cc. of a protoplasmic mass free from dirt and containing only live paramecia could be collected in about one hour's time. Paramecia collected in this way were, as far as could be determined by microscopic examination, normal in activity and appearance and may for all practical purposes be considered as normal animals.

The organisms were usually collected two or three hours before using, and because of the great numbers present they were kept in a beaker at about 15°C. or at room temperature with air bubbling through them to prevent death by suffocation. Before putting the paramecia in the test fluids they were washed one to three times by centrifuging in sterile centrifuge tubes, pouring off the supernatant fluid and shaking in sterile tap-water. In this way the bacteria were reduced to not more than one bacterium to one paramecium.

In some ways it would seem more satisfactory to have compared the various amino-acids at the same molecular concentrations. On the other hand, this would have been impossible in the case of the aminoids, and it would have been none too convenient in the experiments in which combinations of amino-acids were used. That the results do not depend on any of the physical factors associated with a gram molecular solution is evidenced by the similar percentage utilizations of such compounds as phenylalanine and glycocoll, since phenylalanine has a molecule some three times larger than glycocoll. For these reasons, the amino-acids were compared at the same percentage strength.

## PREPARATION OF THE AMINO-ACID SOLUTION

In the beginning experiments aminoids, a mixture of amino-acids containing 9.5 per cent of total nitrogen and 5.7 per cent of amino nitrogen, prepared by the Arlington Chemical Co., Yonkers, New York, were used and in the later work isolated amino-acids[2] were tested. In either case the material to be tested was accurately weighed, some distilled water was added, and the solution sterilized by boiling in a hot-air oven for five minutes. When the solution was cool, the hydrogen-ion concentration was adjusted, by adding a solution of sodium bicarbonate or weak hydrochloric acid in case the solution was alkaline. The optimal pH was between 6 and 7.5, as nearly as could be determined by the use of phenolphthalein and methyl red as indicators. The total volume of the solution was now measured, the washed paramecia were added along with enough sterile tap-water to make the total volume equal 1 cc. per milligram of amino-acid. This procedure resulted in a 0.1 per cent solution, including the paramecia which represented about 4 cc. of protoplasm in each 100 cc. Enough of this solution to yield 60 cc. of supernatant fluid was centrifuged, the supernatant fluid put into a sterile flask and heated to 45°C. for a few minutes, in order to kill the few paramecia that had not been thrown down by the centrifuge. This supernatant fluid served as the two controls, one-half, or 30 cc., was set aside at room temperature as a bacterial control, while the other half, after the addition of thymol, was set aside to be tested at the end of the experiment. This second control, therefore, represented a sample of the material at the beginning of the test, and the first control represented a similar sample subject to the action of the bacteria present. The bacterial control was used throughout all the experiments, although it was demonstrated that the bacteria present were able to change noticeably only the aminoid solution. The single amino-acids or mixtures of these isolated amino-acids were not noticeably affected by the bacteria present during the twelve-hour test period.

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[2] The writer has not tested the purity of these amino-acids. The tryptophane was made here in the organic chemistry laboratory and presented to me by Dr. P. F. Clark, of the Bacteriology Department. I am indebted to Dr. S. Lepkovsky, of the Zoölogy Department, for making the tyrosin. The remaining amino-acids were purchased from the Special Chemicals Co., Highland Park, Illinois. Dr. R. W. Burleigh, the chief chemist of this company, has written me the following: "We take as our criterion of purity a Van Slyke amino nitrogen determination and when this comes within two-tenths of one per cent of the theoretical, the material is approved. The methods of manufacture are such that only mere traces of other materials could be present and the cost of making quantitative analysis of such high priced material is prohibitive."

The original 0.1 per cent amino-acid solution with the paramecia in it was now stirred thoroughly, a sample was removed for counting, and the remainder was divided by means of a sterile graduate cylinder into 40-cc. samples, which were each set aside in sterile 250-cc. Erlenmeyer flasks to be tested at the end of the experiment. The usual test period was twelve hours, except that when anaesthetics were used it was six hours.

The method for counting the paramecia consisted in diluting 1 cc. of the test sample to 100 cc. and counting the paramecia in 0.2 cc. of the diluted sample, by means of a microscope with a mechanical stage attached. To facilitate counting, the paramecia were stained with a dilute Lugol's solution of iodine. The average count of six 0.2-cc. samples was obtained at the beginning and at the end of the experiment, and from this figure the number present in the original test flasks was calculated. A 0.2-cc. sample of the 1-100 dilution usually showed 140 to 170 paramecia per cubic centimeter, or equivalent to 70 to 85 thousand in each cubic centimeter of the original test solution. The tips of all the pipettes used in pipetting paramecia were cut off, in order to give a larger opening and allow a more uniform sample to be asperated into the pipette. The error in a method such as described above for the counting of paramecia is not in the actual counting itself, but in the difficulty encountered in obtaining a uniform distribution of the paramecia in the solution and in transferring by means of a pipette a representative sample of this solution to a slide. Actual calculation based on one hundred samples of 0.2 cc. each taken at random from the experimental data has shown the error of counting to be about 10 per cent. This error has been reduced to some extent by using the average of the twelve samples counted in each test as mentioned above.

## METHOD OF TESTING THE AMINO-ACIDS

The method used throughout these experiments for testing the amino-acids was, with slight modifications, the Henriques-Sörensen formoltitration method as described by Hawk ('23). The sodium hydroxide used was a N/70 solution which was standardized with N/10 hydrochloric acid every four to six weeks. This NaOH solution was kept in a large glass bottle and siphoned through glass tubing into the burette as needed. All the air that entered the supply bottle and burette passed over soda lime; in this way the dilute NaOH solution was kept approximately N/70 normal at all times. The burette used was standardized and graduated to be read in one-hundredths of a cubic centimeter and by the use of a glass bead in a rubber pinch-cock one-fiftieth of a cubic centimeter could easily be withdrawn.

As already explained, the experiments here consisted of keeping paramecia in 0.1 per cent solution of the various amino-acids and determining the amount of the amino-acids metabolized by the paramecia. This procedure has made necessary the testing of, 1) a sample of the solution taken at the beginning of each test, as represented by the second control described above; 2) the testing of a bacteria control, and, 3) samples taken from the test flask at the end of the test. In order to remove the ammonia given off by the paramecia during the test, 30 cc. of the amino-acid solution in which they had lived were shaken for three minutes in large test-tubes with 3 grams of permutit. The slight turbidity from the permutit made the end point of titration easier to read, therefore the controls, although ammonia-free, were likewise shaken with permutit. The removal of certain amino-acids by permutit is discussed under results. By means of a standardized pipette, 10 cc. of the ammonia-free amino-acid solution was placed in a 150-cc. Erlenmeyer flask, three drops of phenolphthalein were added and enough of the NaOH solution run in to give a faint pink color. Immediately 2 cc. of formalin, likewise made faintly pink to phenolphthalein, were added, the acidity thus produced was titrated with the N/70 NaOH and the readings recorded. The average of two duplicate samples was taken to represent the amino-acid present and is referred to as a test in the tables. The amount of the NaOH used in titrating the controls was compared with the amount used in titrating each test sample and the percentage decrease of amino-acid in the test samples thereby determined. This percentage decrease in the amino-acids is considered as the amount utilized by the paramecia. In case of aminoids, which, unlike isolated amino-acids, are utilized by bacteria, the per cent used by the bacteria was obtained, by comparing the two controls as previously described, and this per cent was deducted from the total amount used in order to get the actual amount metabolized by the paramecia. This point is discussed in detail under the next section.

TABLE 1

*Data showing the percentage decrease in a 0.1 per cent solution of the various amino-acids indicated at the end of a twelve-hour test. The figures represent the mean average of ten tests for each amino-acid*

| AMINO-ACIDS | NUMBER OF PARAMECIA PER CUBIC CENTIMETER | PER CENT UTILIZATION | CALCULATED MILLIGRAMS USED BY 100,000 PARAMECIA PER CUBIC CENTIMETER | STANDARD DEVIATION |
|---|---|---|---|---|
| Alanine | 62,400 | 9.7 | .155 | .0265 |
| Arginine | 69,200 | 11.0 | .159 | .0113 |
| Aspartic acid | 65,000 | 16.3 | .251 | .0311 |
| Cysteine hydrochloride | 79,500 | 20.9 | .263 | .0572 |
| Glutamic acid | 69,700 | 9.2 | .132 | .0426 |
| Glutamic acid hydrochloride | 67,500 | 29.4 | .436 | .0458 |
| Glycocoll | 72,000 | 6.9 | .096 | .0136 |
| Leucine | 76,700 | 9.2 | .120 | .0322 |
| Phenylalanine | 80,600 | 6.2 | .077 | .0063 |
| Tryptophane | 73,000 | 7.0 | .096 | .0156 |
| Tyrosin | 70,600 | 12.5 | .177 | .0241 |
| Mixture[1] | 72,100 | 34.1 | .473 | .0584 |

[1] This mixture consisted of equal parts of alanine, aspartic acid, cysteine hydrochloride, glutamic acid, glutamic acid hydrochloride, glycocoll, leucine, phenylalanine, tryptophane, and tyrosin.

## RESULTS

The results obtained throughout these experiments are based upon the per cent reduction of titratable amino-acids when subjected to the action of paramecia for six to twelve hours at room temperature of about 25°C. In order to have a clearer understanding of the rate at which the various amino-acids are used, all the tables are calculated on 100,000 paramecia per cubic centimeter. Table 1 shows the data for the individual amino-acids, while table 2 gives the relation of each amino-acid to all the others tested. Since 1 mg. of

## TABLE 2

*Data showing the mean difference (md.) and the odds that any given amino-acid is used more than any other amino-acid with a smaller mean average. Example: the odds = 14 to 1 that alanine is used more than glutamic acid or the odds = 14 to 1 that glutamic acid is used less than alanine. For details of the calculations see alanine.*

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Phenylalanine**<br>σ = .0063 | | | | | | | | | | | |
| **Tryptophane**<br>σ = .0156 | md. 019<br>odds =<br>344–1 | | | | | | | | | | |
| **Glycocoll**<br>σ = .0136 | md. 019<br>odds =<br>908–1 | | | | | | | | | | |
| **Leucine**<br>σ = .0322 | md. 043<br>odds =<br>555–1 | md. 024<br>odds =<br>37–1 | md. 024<br>odds =<br>37–1 | | | | | | | | |
| **Glutamic acid**<br>σ = .0426 | md. 055<br>odds =<br>555–1 | md. 036<br>odds =<br>61–1 | md. 036<br>odds =<br>61–1 | md. 012<br>odds =<br>4–1 | | | | | | | |
| **Alanine**<br>σ = .0265 | md. 078<br>odds =<br>100,000–1 | md. 059<br>odds =<br>20,000–1 | md. 059<br>odds =<br>20,000–1 | md. 035<br>odds =<br>216–1 | md. 023<br>odds =<br>14–1 | | | | | | |
| **Arginine**<br>σ = .0113 | md. 082<br>odds ><br>100,000–1 | md. 063<br>odds ><br>100,000–1 | md. 063<br>odds ><br>100,000–1 | md. 039<br>odds =<br>344–1 | md. 027<br>odds =<br>22–1 | md. 004<br>odds =<br>2–1 | | | | | |
| **Tyrosin**<br>σ = .0241 | md. 100<br>odds ><br>100,000–1 | md. 081<br>odds ><br>100,000–1 | md. 081<br>odds ><br>100,000–1 | md. 057<br>odds =<br>3300–1 | md. 045<br>odds =<br>163–1 | md. 022<br>odds =<br>61–1 | md. 018<br>odds =<br>37–1 | | | | |
| **Aspartic acid**<br>σ = .0311 | md. 174<br>odds ><br>100,000–1 | md. 155<br>odds ><br>100,000–1 | md. 155<br>odds ><br>100,000–1 | md. 131<br>odds ><br>100,000–1 | md. 119<br>odds =<br>100,000–1 | md. 096<br>odds =<br>100,000–1 | md. 092<br>odds =<br>100,000–1 | md. 074<br>odds =<br>33,000–1 | | | |
| **Cysteine HCl**<br>σ = .0572 | md. 186<br>odds =<br>100,000–1 | md. 167<br>odds =<br>100,000–1 | md. 167<br>odds =<br>100,000–1 | md. 143<br>odds =<br>50,000–1 | md. 131<br>odds =<br>25,000–1 | md. 108<br>odds =<br>10,000–1 | md. 104<br>odds =<br>5,000–1 | md. 086<br>odds =<br>1428–1 | md. 012<br>odds =<br>3–1 | | |
| **Glutamic acid HCl**<br>σ = .0458 | md. 359<br>odds ><br>100,000–1 | md. 340<br>odds ><br>100,000–1 | md. 340<br>odds ><br>100,000–1 | md. 316<br>odds ><br>100,000–1 | md. 304<br>odds ><br>100,000–1 | md. 281<br>odds ><br>100,000–1 | md. 277<br>odds ><br>100,000–1 | md. 259<br>odds ><br>100,000–1 | md. 185<br>odds ><br>100,000–1 | md. 173<br>odds ><br>100,000–1 | |
| **Mixture**<br>σ = .0584<br>mean .483 | md. 406<br>odds ><br>100,000–1 | md. 387<br>odds ><br>100,000–1 | md. 387<br>odds ><br>100,000–1 | md. 363<br>odds ><br>100,000–1 | md. 351<br>odds ><br>100,000–1 | md. 328<br>odds ><br>100,000–1 | md. 324<br>odds ><br>100,000–1 | md. 306<br>odds ><br>100,000–1 | md. 232<br>odds ><br>100,000–1 | md. 220<br>odds ><br>100,000–1 | md. 047<br>odds =<br>49–1 |
| | **Phenylalanine**<br>mean .077 | **Glycocoll**<br>mean .096 | **Tryptophane**<br>mean .096 | **Leucine**<br>mean .120 | **Glutamic acid**<br>mean .132 | **Alanine**<br>mean .155 | **Arginine**<br>mean .159 | **Tyrosin**<br>mean .177 | **Aspartic**<br>mean .251 | **Cysteine HCl**<br>mean .263 | **Glutamic acid, HCl**<br>mean .436 |

amino-acid was used per cubic centimeter, the tables may be read in milligrams or percentage. The amino-acids studied are discussed in alphabetical order as follows:

*Aminoids.* The composition of the aminoids has been previously discussed. The small cost of this material has made it possible to try a large number of preliminary tests. These tests for the most part were done in the early part of the work and consisted chiefly in refining the technique of titration, removal of ammonia, and controlling the bacteria factor. Without this preliminary work, the experiments on the expensive isolated amino-acids would not have been possible.

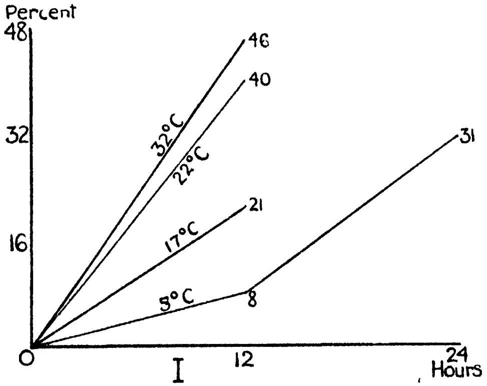
Ammonia is produced by paramecia and must be removed before the amino-acids are titrated, because the Henriques-Sörensen method gives both ammonia and amino-acids. In these experiments the ammonia was removed by permutit, as recommended by Folin and Bell ('17). When ammonia is removed from aminoids by permutit, complications arise, because some of the amino-acids in the aminoid solution are likewise removed. Since the controls were also shaken with permutit, a similar amount of amino-acids would be expected to be removed from the controls, but the amount removed has been found to vary somewhat with the percentage of ammonia present. A number of experiments were therefore tried by adding ammonium chloride to a 0.1 per cent aminoid solution in amounts similar to the ammonia given off by the paramecia. This aminoid-and-ammonium-chloride solution, as well as samples of the original 0.1 per cent aminoid solution, were shaken in a similar quantity of permutit and the amino-acid titration of the two compared. The results showed that about 5 per cent more amino-acids had been removed from an ammonia-free aminoid solution than from an aminoid solution containing an amount of ammonium chloride equivalent to 1.5 cc. of N/70 NaOH in each 10 cc. It was also found that the larger part of the amino-acids that permutit would remove could be extracted by shaking the aminoid solution twice with permutit; therefore, in the results here reported, the aminoid solution was treated twice with permutit before sterilization. By this method the error in the ammonia factor is somewhere around 3 per cent, which is well within the variability of the results obtained. It should be mentioned that all attempts to remove the ammonia, by bubbling air through an alkaline solution of a 0.1 per cent aminoids, have resulted in a destruction of the titratable amino-acids present.

It was found in the beginning part of the work that bacteria have surprising power to reduce the titratable amino-acids in a solution of aminoids. This factor was controlled by using supernatant fluid from centrifuged samples of the test flasks. This supernatant fluid, when centrifuged twice at a rate sufficient to throw down the paramecia and then heated to 45°C. to kill the few paramecia remaining, still contained live bacteria. Agar and gelatin plates that had been inoculated at the beginning of the experiment with dilutions from the control and test flasks showed that the bacteria content of each was about the same. It was found that the number of bacteria usually were less than 50,000 per cubic centimeter and in a few cases less than 10,000. At the end of the experiment, the control flask showed an increase in bacteria, while the test solution remained about the same and in some cases showed a marked decrease. These results were interpreted to mean that the paramecia in the test solution had digested the bacteria at a rate equal to, or greater than, the rate of reproduction of the latter. Similar observations on the reduction of bacteria by paramecia have been reported by Woodruff and Baitsell ('11). From these observations it seems evident that the controls used were adequate in controlling the part played by bacteria in these experiments. The per cent utilized by bacteria was determined by comparing the bacteria control with the original solution. This percentage, representing the part played by bacteria, was then subtracted from the total utilization of bacteria and paramecia and the result obtained taken to represent the utilization by the paramecia.

The amount of aminoids utilized by paramecia is more than that with any other amino-acid solution tried, but the variability is decidedly marked. Within the several flasks of any one series the variability is small as compared with the isolated amino-acids, but the variability is large when one series of tests is compared to any other similar series, even though the results were all calculated on a standard basis of 100,000 paramecia per cubic centimeter for a test period of twelve hours. This variability in many instances was more than 50 per cent.

Since constant results were obtained with the various samples within a single series, an opportunity was afforded to study some factors influencing the rate of utilization. The influence of temperature was determined by placing 0.1 per cent aminoid solutions in control and culture flasks at various temperatures. In each series two of these flasks were placed at each of the four temperatures, 5°, 17°, 22°, and 32°C. At the end of twelve hours, the amount of amino-acids was determined. Five series representing ten tests at each temperature have been plotted in graphic form in figure 1. The increased rate of utilization seen when the 5°C. flask was put at a room temperature of 22°C. indicates that the paramecia were not killed after being at a temperature of 5°C. for twelve hours. The utilization at 32°C. was extremely high, being 46 per cent.

The effect of anaesthetics upon the utilization of aminoids was tried and the results obtained compared with the normal. Air that had bubbled through ether and chloroform was passed through the test samples. Both of these substances produced marked anaesthesia, and if carried too far killed the paramecia. By microscopic examination from time to time during the experiment the anaesthetic could be controlled so as to keep the paramecia in a depressed state, but still motile. The degree of anaesthesia affected directly the rate of utilization, reducing it in the case of chloroform to 17 per cent and in ether, to 8 per cent, as compared to 25 per cent in the normal (fig. 2). The air was bubbled through the solution at the rate of one small bubble per second, and ether, being more volatile, gave the deeper anaesthesia under these conditions, and therefore the greater decrease in utilization. Ethylene gas was bubbled through the solution two or three times faster than ether or chloroform without producing any marked microscopic signs of slowing the movement of the paramecia. In a few preliminary tests a stimulating effect was observed, but this could not again be produced. That ethylene may at times have a slight stimulating effect on oxygen consumption can be seen in the graphs of Leichsenring ('25), although this author concludes that the effect is slight or absent. In the experiment reported here there was generally a slight decrease in utilization, which may have been due to a slight toxicity instead of anaesthesia, as is seen in the case of nitroglycerin when used in 1–50,000 dilution, as shown in figure 2.



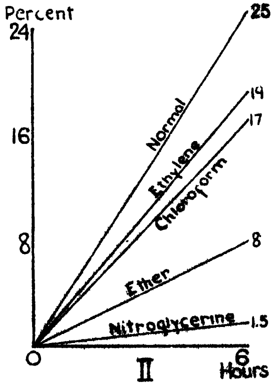


Fig. 1 Curves showing the per cent of utilization, at various temperatures, of about a 0.1 per cent aminoid solution by 50,000 paramecia per cubic centimeter.

Fig. 2 Curves showing the decrease in metabolism brought about by the substances indicated. The data are based on 100,000 paramecia per cubic centimeter in about a 0.1 per cent aminoid solution. (The nitroglycerin was used in 1–50,000 solution.)

*Alanine.* The percentage utilization of alanine is quite constant, with a mean average of 15.5 per cent during a twelve-hour test, and a standard deviation of 0.0265. In working out the probability that this result is not due to chance, the method of Student ('08) has been used. This method takes into consideration the pairing of the samples which refer here to the control and the test samples. When the mean average of the difference between two pairs is divided by the standard deviation, a figure is obtained which Student has called Z. The value of Z is obtained from a table (Student, '08, or Love, '24) which gives the odds that these results may occur again. In case of alanine, table 1, the value of Z would be 6—a value which is too high to be read from the tables—but if the value were 3 the odds would be 100,000 to 1 that such a large variation, between the control and test sample, is not due to chance alone. A comparison of alanine with the other amino-acids is shown in table 2. In this table the Student method is likewise used for obtaining the odds, the larger standard deviation, of the two amino-acids being used in obtaining the value Z. As shown in this table, alanine is utilized to about the same extent as arginine, the odds being only 2 to 1 that argine is utilized more.

*Argenine.* The error in the removal of arginine by permutit was worked out as with the aminoids mentioned above. The results obtained were constant as represented by a standard deviation of 0.0113. The rate of utilization was 15.9 per cent, which places arginine between alanine and tyrosin; however, the odds are only 2 to 1 that arginine is used more than alanine and 37 to 1 that tyrosin is used the more (tables 1 and 2).

*Aspartic acid.* The results obtained show a mean rate of utilization of 25.1 per cent, during a twelve-hour test, and quite a large variation between the tests. The standard deviation is also quite large, being 0.0311, but relatively small when compared with the mean. Aspartic acid ranks between tyrosin and cysteine hydrochloride in its rate of utilization. The odds are 33,000 to 1 that aspartic acid is used more than tyrosin and 3 to 1 that cysteine hydrochloride is utilized more than aspartic acid.

*Cysteine hydrochloride.* The rate of utilization is similar to aspartic acid, being 26.3 per cent during a twelve-hour test, which is higher than any other amino-acid except glutamic acid hydrochloride. As represented in table 2, the odds are only 3 to 1 that cysteine hydrochloride is utilized more than aspartic acid, but 100,000 to 1 that it is metabolized less than glutamic acid hydrochloride. It was not found to be removed by commercial permutit, but freshly cleaned permutit in some cases seemed to remove it slightly.

*Cystine.* This amino-acid is practically insoluble in water, only a small fraction going into solution. Permutit will remove cystine in small amounts, which may have obscured a small rate of utilization, if present. In a series of tests it was found that cystine was not used in amounts sufficient to be detected. The constancy of the results obtained indicated as nearly as can be calculated that if cystine had been utilized at the rate of 1 per cent during a twelve-hour test it would have been detected. Since cystine was not found to be used and cysteine hydrochloride was readily metabolized, it seems evident that paramecia cannot change cystine to cysteine, as had been reported in higher animals by Lewis, Updegraff, and McGinty ('24).

*Glutamic acid.* The results obtained with glutamic acid as shown in table 1, were quite variable as represented by a standard deviation of 0.0426. The mean rate of utilization is relatively small, being 13.2 per cent during a twelve-hour test, which places it between leucine on the one side and alanine on the other (table 2). The odds are 4 to 1 that glutamic acid is metabolized more than leucine, and 14 to 1 that it is less than alanine.

*Glutamic acid hydrochloride.* It is interesting to note that the hydrochloride form of glutamic acid shows a mean percentage of utilization of 43.6 per cent during a twelve-hour test, or more than three times that of glutamic acid and greater than any other amino-acid. Since the tests were carried out in the same manner in the two cases, it seems likely that some specific factor or factors are present which enables glutamic acid hydrochloride to be utilized more readily by paramecia. The standard deviation, like glutamic acid, is high, being 0.0458. When compared with the mixture of amino-acids, the odds are 49 to 1 that the mixture is used more; on the other hand, the odds are more than 100,000 to 1 that glutamic acid hydrochloride is utilized more than cysteine hydrochloride (table 2).

*Glycocoll.* About forty different tests have been conducted with glycocoll, and ten of these showing a mean average of 9.6 per cent during a twelve-hour test are given in table 1. In respect to the other amino-acids, glycocoll ranks between phenylalanine and leucine, with odds of 900 to 1 that it is metabolized more than phenylalanine and 37 to 1 that it is used less than leucine. Glycocoll is removed by permutit, so this error had to be taken into account and was worked out as with the aminoids previously discussed. The removal of ammonia by bubbling air through a 0.1 per cent solution of glycocoll in a 1 per cent solution of sodium bicarbonate at 70°C. has given results comparable to those obtained by permutit, but this method of removing ammonia is uncertain, due to the fact that a part of the glycocoll may be destroyed. In some cases, after removing the ammonia by permutit, the test samples were passed through a microfilter, which removed the protein fragments of dead paramecia and allowed only the amino-acid to pass through. This clear solution was tested for nitrogen by a microchemical method similar to that described by Folin and Farmer ('12). The control samples were similarly shaken in permutit, likewise filtered and tested, and the results compared to the test samples. A series of ten tests shows a mean average of 7.2 per cent, as compared to 9.6 per cent by the formol-titration method. It is to be noted that this method does not give as high a percentage of utilization as does the formol-titration method shown in table 1. One explanation of this difference may be due to the fact that paramecia excrete other nitrogenous products beside ammonia, and these would influence the results obtained by the total nitrogen method, and therefore lessen the total rate of utilization. At least one nitrogenous excretory product other than ammonia, namely, uric acid, has been reported (Griffith, '89, and Howland, '24).

*Histidine.* The removal of ammonia by bubbling air through the test samples of a 0.1 per cent solution of histidine, made alkaline with sodium bicarbonate or magnesium oxide, has been found to destroy the histidine. With permutit histidine is removed from solution with remarkable ease, as much as two-thirds of a 0.1 per cent solution being removed by the usual amount of permutit used in removing ammonia. As nearly as could be determined, the utilization of histidine is around 10 to 15 per cent, but the correction for the histidine removed by the permutit is so great that the results are somewhat confusing and are therefore not given in a table.

*Leucine.* This amino-acid is used only moderately. When compared to the others, it ranks above tryptophane and glycocoll and below glutamic acid. The odds are 37 to 1 that it is utilized more than glycocoll or tryptophane and 4 to 1 that it is less than glutamic acid (table 2). The rate of utilization is quite variable, as shown by a standard deviation of 0.0322 and a mean average of 12 per cent during a twelve-hour test (table 1).

*Lysine.* Like histidine, lysine is readily removed by permutit, and therefore its rate of utilization is uncertain. As nearly as could be determined, lysin picrate is utilized about 5 per cent, but the results are too uncertain to place this amino-acid in a table or to compare it to other amino-acids.

*Phenylaline.* The rate of utilization of phenylaline, as shown in table 1, is only 7.7 per cent during a twelve-hour test, being the least of all the amino-acids tested, and likewise the standard deviation, which is 0.0063, is the least. When compared to glycocoll or tryptophane, the odds are 900 to 1 in favor of glycocoll being utilized more, and 344 to 1 in favor of tryptophane (table 2).

*Tryptophane.* The rate of utilization of tryptophane was the same as that of glycocoll, with a mean average of 9.6 per cent during a twelve-hour test, but the standard deviation was higher, being 0.0156, as shown in table 1. When compared to phenylalanine, the odds are 344 to 1 in favor of tryptophane being more readily used. On the other hand, the odds are 37 to 1 that leucine is utilized more than tryptophane (table 2). Both commercial tryptophane and that made in the chemistry laboratory here were tried and found to give comparable results.

*Tyrosin.* Due to the slight solubility of tyrosin, 0.05 per cent solutions were tested and the data calculated on a 0.1 per cent solution basis, so it could be compared with the results obtained for the other amino-acids. The mean average is 17.7 per cent during a twelve-hour test and the standard deviation is 0.0241. In table 2 tyrosin is placed between arginine and aspartic acid. The odds are 37 to 1 that tyrosin is utilized more than arginine and 33,000 to 1 in favor of aspartic acid when compared to tyrosin. Like tryptophane, both commercial and tyrosin prepared in the laboratory were used with comparable results.

*Mixture of amino-acids.* This mixture contained equal parts of ten amino-acids consisting of alanine, aspartic acid, cysteine hydrochloride, glutamic acid, glutamic acid hydrochloride, glycocoll, leucine, phenylalanine, tryptophane, and tyrosin. A 0.1 per cent solution was used and the tests carried out as with the other amino-acids. The results show this mixture to be utilized more than any of the amino-acids tested, having a mean average of 48.3 per cent (table 1). The variability in the rate of utilization is quite high as represented by a standard deviation of 0.0584. When compared to glutamic acid hydrochloride (table 2), the odds are 49 to 1 in favor of the mixture being utilized more. It was thought that the high rate of utilization of this mixture might be due to the influence of glutamic acid hydrochloride and cysteine hydrochloride, which have been shown to be utilized more than any of the other amino-acids. In a second series, consisting of six tests, these two amino-acids were left out and the other eight amino-acids mentioned above were used. The results obtained show a similar rate of utilization as when glutamic acid hydrochloride and cysteine hydrochloride were present, which indicates these two amino-acids are not responsible for the high rate of utilization obtained with an amino-acid mixture. As to why a mixture of amino-acids should be utilized more than any one of the amino-acids taken separately is an interesting speculation. It may be due to a slight summation effect of all the amino-acids used in the mixture or to the stimulating effect of certain amino-acids or to a combination of factors which are not at all clear.

*Ammonia.* The amount of ammonia produced by the paramecia was determined by the formol-titration method described for amino-acids. Since N/70 NaOH was used in these determinations, the amount of ammonia is reported in cubic centimeters of N/70 NaOH. The results based on the average of ten tests for each solution show that the amount of ammonia given off by 100,000 paramecia during a twelve-hour period in tap-water was 0.64 cc., in glycocoll 1.04 cc., in tyrosin 1.27 cc., and in a mixture of ten amino-acids 2.25 cc. When the rate of utilization of these amino-acids (glycocoll 9.6 per cent, tyrosin 17.7 per cent, and a mixture of ten amino-acids 48.3 per cent) is compared to the ammonia produced, it will be seen that the greater the metabolism the greater the amount of ammonia produced.

## DISCUSSION

As has been discussed under the preparation of the amino-acid solution, the amino-acids used in these experiments were, with the exception of tyrosin and tryptophane, commercially prepared. If there were impurities in these amino-acids, it is very unlikely that the amount of these impurities was sufficiently great to materially alter the results obtained. It should be borne in mind that the experiments here reported are not based on growth and maintenance determinations, but upon the rate of utilization during a twelve-hour test; therefore, traces of other amino-acids or non-toxic substances would not noticeably influence the results. Tests made on the comparison of both commercial and laboratory preparations of tyrosin and of tryptophane failed to show any difference between the two products.

It was observed by microscopic examinations of the living paramecia, made from time to time during the test periods, that the animals appeared normal in all the amino-acids but cystine. The fact that the paramecia appeared starved when in the cystine solution gives further evidence that the other amino-acids were utilized for energy requirements.

The part played by bacteria and dead paramecia is a question of considerable importance. Samples of the controls and test solutions when plated on agar and gelatin plates were found to contain a similar number of bacteria. The utilization by the bacteria was determined, and in this way the true utilization of the aminoid solution by the paramecia has been very closely calculated, as has been discussed under aminoids. Under the conditions of these experiments, the bacteria present did not noticeably change any of the isolated amino-acid solutions. The bacteria and paramecia have been counted, and these counts show usually less than one bacterium to one paramecium in the test samples. A further reduction of the bacteria by washing the paramecia repeatedly in sterile water is possible, but dangerous to the normal state of the paramecia. In consideration of these facts, it seems the controls used were as adequate as possible and that the error in the results due to the influence of bacteria on isolated amino-acid solutions is for all practical purposes negligible. Aside from bacteria, protein substances from dead paramecia are present. Calculations based upon 300 counts at the beginning and 300 counts at the end of the same experiments have shown that an average of 1300 paramecia per cubic centimeter, or about 1.5 per cent of the paramecia, died during the test. Samples of 10 cc. containing this number of paramecia per cubic centimeter when decomposed by boiling in concentrated sulphuric acid or digested by trypsin did not produce enough titratable amino-acid to give a test by the Henriques-Sörensen formol method. On this basis, it seems unlikely that the production of amino-acids from the dead paramecia was of any great significance. It should, however, be understood that the rate of reproduction during the test is unknown, and therefore it is not unlikely that considerable more paramecia may have died than those shown by the counts taken at the beginning and at the end of the test.

The removal of the ammonia from the test solution has caused more difficulty than any other factor encountered. The permutit method of removing ammonia as described by Folin and Bell ('17) has given the most satisfactory results. According to Whitehorn ('23), lysin, histidine, arginine, and traces of tyrosin were removed by permutit. With the possible exception of tyrosin, these amino-acids have been found to be removed by permutit and in addition glycocoll and cystine were removed. The different preparations of permutit were found to differ in the amount of these amino-acids they would remove, as is demonstrated by the fact that cysteine hydrochloride was removed only when freshly recleaned permutit was used.

Throughout the work an explanation of why one amino-acid was used more than another amino-acid has been sought for, but not discovered. Some of the factors suggested have been solubility, chemical structure, and increased metabolism brought about by the reproduction of the paramecia. A study of figure 3 and table 2 shows that solubility is not a major factor, if of any significance, because tyrosin which is practically insoluble is metabolized more than the more soluble amino-acids, phenylalanine, tryptophane, glycocoll, leucine, glutamic acid, alanine, and arginine. A careful study of the tables has failed to show any relationship existing between the chemical formulae and the rate of utilization. The reproduction of the paramecia in the tests reported here is probably not of any great significance, because an attempt was made to culture the paramecia in a 0.1 per cent solution of single isolated amino-acids without success. Evidence that paramecia can use the nitrogen of amino-acids for growth has been presented by Peters ('21). There was found to be a gradual decrease in the number of organisms, and at the end of two weeks all were dead, even though fresh medium was added every two or three days. That reproduction can occur was evident by the fact that in a few instances paramecia were seen dividing after being twelve hours in the amino-acid solutions. In 1 per cent amino-acid solutions the paramecia were dead in glutamic acid, glutamic acid hydrochloride, histidine, aspartic acid, and a mixture of amino-acids at the end of one hour, but alive in all the other amino-acids. This toxicity does not seem to be correlated with the rate of utilization in 0.1 per cent solutions.

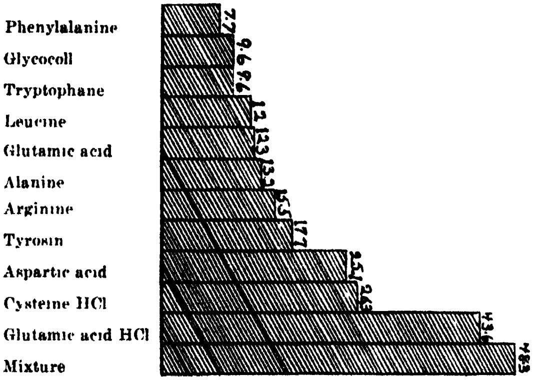


Fig. 3 Graph showing the percentage utilization of a 0.1 per cent solution of amino-acids during a twelve-hour test period based on 100,000 paramecia per cubic centimeter.

The odds as expressed in table 2 should be regarded as a mathematical expression of the relation that one amino-acid bears to another. For example, the odds are 14 to 1 that alanine is utilized more than glutamic acid, which means if fifteen tests were made, comparing the utilization of these two amino-acids, one could reasonably expect alanine to be utilized the more in about fourteen cases and glutamic acid more than alanine in about one case out of the fifteen.

## SUMMARY

1. A method has been described whereby an amount of paramecia equal to 15 cc. of protoplasm has been collected within a few hours.

2. Paramecia have the power to decrease the amino-acids present in a 0.1 per cent solution of both isolated and mixtures of amino-acids as represented by Henriques-Sörensen formol method of testing amino-acids. This decrease has been considered to be due to the metabolism of the amino-acids by the paramecia.

3. Tests run at 5°, 17°, 22°, and 32°C. show that an increase in temperature causes an increase in the amount of amino-acids utilized (fig. 1).

4. A depressing effect and a corresponding decrease in the rate of utilization of aminoids has been produced by chloroform, ether, ethylene, and nitro-glycerin (fig. 2).

5. The rate of metabolism of fourteen isolated amino-acids has been studied and eleven of these have been worked out in detail and presented in tables. These various amino-acids have been compared and the odds that one amino-acid is used more or less than any other amino-acids has been determined (table 2).

6. The order expressed in per cent in which the amino-acids were found to be metabolized were phenylalanine 7.7, tryptophane 9.6, glycocoll 9.6, leucine 12.0, glutamic acid 13.2, alanine 15.5, arginine 15.9, tyrosin 17.7, aspartic 25.1, cysteine hydrochloride 26.3, glutamic acid hydrochloride 45.6, and a mixture, consisting of equal parts of all of these amino-acids except arginine, 48.3 per cent (fig. 3).

7. The greater the rate of utilization of any amino-acid, the greater was the amount of ammonia produced.

8. Evidence that paramecia cannot change cystine to cysteine has been obtained.

9. The influence of bacteria on the results was negligible when isolated amino-acids were tested.

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# DEVELOPMENTAL FACTORS IN TELEOST HYBRIDS

## I. THE EARLY MITOTIC BEHAVIOR IN THE CROSS BETWEEN MENIDIA MENIDIA NOTATA ♀ AND PRIONOTUS CAROLINUS ♂

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TWO PLATES (TWELVE FIGURES)

AUTHOR'S ABSTRACT

When Menidia eggs are fertilized with Prionotus sperm, the Prionotus chromosomes react in the Menidia cytoplasmic medium just as they do in the cytoplasm of Fundulus eggs. There is lagging, non-disjunction, and elimination of chromosomes during the early cell divisions. The mitotic behavior of the Prionotus sperm in the Menidia egg also resembles the behavior of the sperm of Ctenolabrus in the same medium. This behavior which was expected from what was known concerning the mitotic behavior of the reciprocal crosses between Menidia and Fundulus and between Ctenolabrus and Prionotus and other intercrosses between the members of these two groups is regarded as a function of the physical state of the egg cytoplasm during the division phase of mitosis. This physical character forms the earliest differential factor in the development of these hybrids and shows no correlation with the width of the cross.

A comparison of nine teleost crosses, in which both the development and the early mitotic behavior are known, with a rough numerical estimate of the width of the cross brought out the fact that development is most successful in crosses between nearly related species if mitosis is normal and in distantly related crosses if mitosis is abnormal. This indicates that nuclear relationship is also a factor in the development of hybrids.

The work reported here was undertaken to test the hypothesis, suggested by the writer in a former paper(10), that the early behavior of the paternal chromosomes in heterogeneric crosses is due to the physical state of the cytoplasm of the egg. This condition occurred independently of species affinities and was regarded as a valid basis on which the lack of correlation between the results of heterogeneric hybridization and taxonomic relationships might be explained (pp. 409 and 413).

> The nature of the mitotic disturbances which have been described above would indicate that the immediate factor was a physical condition of the egg cytoplasm, probably the normal state of fluidity or viscosity characteristic of the division phase.
>
> . . . . . . the condition itself is no doubt an expression of the specific chemical composition of the egg cytoplasm. This chemical composi-

tion may be more highly specific than the physical state which it conditions, that is, it is conceivable that the egg protoplasms of two species of fish may resemble each other so closely in their physical characters that they may react alike in crossing. In other words, protoplasmic relationships between species are not necessarily correlated with the physical conditions present in their germ cells at corresponding morphological stages.

For the sake of clearness, the facts noted in the studies of fish hybrids which pointed to this conclusion may be briefly restated. In cross-fertilized fish eggs two general sorts of mitosis may occur. One type is normal, the chromosomes align themselves on the equator of the spindle, split, the daughter halves separate cleanly and pass to the poles in straight orderly ranks. The other type is abnormal, the paternal chromatin lags on the spindle and becomes eliminated from the nucleus, or it fails to divide, as in non-disjunction, and is distributed irregularly to the early blastomeres. Among the twelve crosses examined previously, all but one of which were studied by the writer, two sets of reciprocal crosses were found in which the paternal chromosomes reacted normally in the early cell divisions. These were the crosses of Fundulus heteroclitus with Menidia menidia notata and Ctenolabrus adspersus with Prionotus carolinus. Whichever way the cross was made between the two members of either pair, mitosis proceeded normally. That this was an indication of similar cytoplasmic conditions prevailing during mitosis in the eggs of both species was evidenced by the fact that the sperm of Ctenolabrus found the cytoplasm of both Fundulus and Menidia unfavorable to their normal participation in mitosis. Further confirmation of this point was obtained in crossing the egg of Fundulus with the sperm from both of the reciprocating species, Prionotus and Ctenolabrus. The Prionotus sperm behaves in the foreign Fundulus cytoplasm as does the sperm of Ctenolabrus in the same medium, there is lagging, non-disjunction, and elimination as in all of the cases of abnormal mitosis. Further, both Fundulus and Menidia sperm behave normally in the dividing egg of Ctenolabrus. It was also found that Baltzer's results with echinoderm hybrids favored the same interpretation(1).

In view of these facts, the cross Menidia menidia notata ♀ × Prionotus carolinus ♂ seemed to offer an especially critical test of the idea that the eggs of normally reciprocating species would react in the same way to the same sperm or that the sperm of a reciprocating pair would show the same response in any egg. Both of these points could be tested in this cross, the expected answer is the same in both cases. If the hypothesis is correct, such eggs should show a characteristic mitotic disturbance. Since the Fundulus egg reacts unfavorably to the sea-robin sperm, the egg of Menidia should do the same. Put the other way about, since the sperm of Ctenolabrus finds the cytoplasmic medium of the Menidia egg unsuitable to its normal functioning, the sperm of Prionotus should meet with a similar fate in the same egg.

## OBSERVATIONS

The material for this study was obtained from two experiments in which eggs of the little silver-sides, Menidia menidia notata, were fertilized with the sperm of Prionotus carolinus, the common gurnard or sea-robin. The eggs were fixed for first, second, and third cleavage stages in $B_3$. (Saturated aqueous solution of picric acid, 75 cc.; formalin, 15 cc.; glacial acetic acid, 10 cc.; urea, 1 gram.)

Owing to the limited number of eggs, care was taken to conserve the material by orienting the eggs when embedding so that in sectioning the blastoderms the anaphase spindles would be included in the plane of the section. Sections, 8 μ in thickness, may contain an entire spindle in lateral view, but more often one spindle must be sought through two or even three sections. Accurate counts of chromosomes cannot be made from these preparations, although they are well adapted to observations on the size, shape, and general character of these bodies.

Figures 1, 2, and 3, from first, second, and third cleavage spindles, respectively, show very typical cases of the sort of mitosis which one finds in these hybrid eggs. The lagging chromosomes and the undivided clumps of chromatin at the equator of the spindle reproduce the conditions already met with in other crosses, especially in Menidia ♀ × Ctenolabrus ♂ and Fundulus ♀ × Prionotus ♂. Since this is what was expected in this cross, the observations were carried out with extreme care in order to eliminate all effects of bias.

There are four types of behavior exhibited by the dividing chromosomes in these eggs, three of which are indicative of a deviation from the normal course of mitosis. In figures 2 and 3 those chromosomes that are gathered at the poles have undergone the usual process of division characteristic of self-activated eggs. In addition, there are some belated elements on their way to the poles. These will eventually be drawn into the nuclei of the daughter cells. In a few eggs the mitotic abnormalities are entirely of this less pronounced sort, but this is relatively infrequent. Figures 4 and 5 show this condition. Figures 2 and 3 show undivided bodies which, from their dimensions, seem to be undivided chromosomes that are being drawn into the polar area. In most of the mid-anaphase spindles undivided chromosomes lying at or very near the equator of the spindle can be demonstrated. There is evidence that some at least of these bodies never reach either daughter nucleus and are eliminated. Figures 8 and 9 indicate this. In figure 8 the spindle fibers have disappeared except through the equatorial region. There, where the new cell wall will appear, they persist for some time. Definite chromatin bodies lie in this plane which I interpret as eliminated chromosomes. That they are not yolk granules or other deeply staining cytoplasmic inclusions is certain, since these latter bodies very rarely invade the area of the spindle in this cross. The telophase vesicles, owing to their slight affinity for nuclear dyes, are almost invisible. In the egg from which figure 9 was drawn the chromosomes were just beginning to form vesicles, but were too lightly stained to give a definite picture.

While lagging occurs in practically all of the eggs, it varies in amount. In a careful examination of eighty spindles from eggs of the first three cleavages only eight were found which showed no traces of its occurrence. One of these was in a one-celled egg and the other seven were from two-celled eggs. Of the latter, five belonged to eggs in which but one cell was affected, only one two-celled egg showed no evidence of the abnormality. In all of the thirty-seven spindles from four-celled eggs retarded division was present. These doubtful cases cannot be accepted as conclusive evidence that lagging was absent altogether. In interpreting these figures one must keep in mind the natural course of nuclear division in self-activated eggs. It will be remembered that the anaphase is of very short duration. The chromosomes mount to the poles rapidly and there immediately undergo vesiculation and combine to form the daughter nuclei. If the lagging was not extensive, but like that shown in figures 4 and 5, it is conceivable that all evidence of its occurrence would have disappeared by the time the late anaphase or early telophase was reached. At any rate, the number of eggs from which lagging seems to be absent are too few to outweigh the fact of its occurrence in the large majority. And in those eggs which are definitely abnormal the condition varies between the extremes shown in figures 4 and 5, on the one hand, and 2, 3, and 7, on the other. Figure 11 shows a spindle in which all of the chromosomes but one have been drawn into the polar groups, and this one seems to have been arrested just in the midst of the separation of its daughter components. Whether this particular element would have been eliminated or not is impossible to decide. Figure 12 is a portion of a late anaphase spindle in which only two chromosomes have definitely been eliminated. Undoubtedly, more would be extruded from spindles such as are shown in figures 6 and 7.

While the amount of chromatin extruded after any one cleavage is small, the net result must be considerable, since we know that it takes place at each of the first three divisions. The number of chromosomes lost from the nucleus has not been determined. The Menidia egg is not as favorable material for counting chromosomes as another egg which could be obtained in greater numbers would be. Matsui(5) has studied a similar phenomenon in the cross Echinarachnius parma ♀ × Arbacia punctulata ♂.[1] He found that elimination occurred during the first and second segmentation stages only. His counts indicated that four or five chromosomes were missing from the polar groups of the first two anaphases. His lateral views of such spindles show more retarded chromosomes than these counts would lead one to expect. Evidently retardation does not always result in elimination. My observations indicate that the number of chromosomes eliminated varies among the individuals of one cross.

It has been found, especially in the Fundulus and Menidia crosses, that the parental chromosomes maintain their original spatial relations during the first few cell-divisions. The lagging chromatin which characterizes the eggs of Menidia that are fertilized by the sperm of Prionotus is definitely limited to one side of the spindle. This is not as evident in the drawings as it is from a study of the sections themselves. It is taken to mean that the chromosomes involved originated from but one of the two parents. Evidence from other crosses indicates that it is the paternal chromatin that behaves atypically.

No other signs of nuclear abnormality have been seen during this study. The prophase nuclei are entirely characteristic, as are the metaphase plates of chromosomes. Figures 1 and 10, which are typical of very early anaphases, each show bodies at the equator of the spindle which are apparently resistant to splitting and division, but there is no other earlier condition observable that foretells this behavior.

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[1] Fry, Henry J. (Biol. Bull., vol. 53, Sept., 1927), has reported observations on the cross-fertilization of enucleated Echinarachnius eggs by Arbacia sperm which are significant in this connection. The failure of enucleated Echinarachnius eggs inseminated with Arbacia sperm to develop beyond the blastula stage may be attributed to the abnormal behavior of Arbacia chromosomes in the cytoplasmic medium of the Echinarachnius egg as described by Matsui, and their consequent inability to maintain the nuclear organization necessary to normal developmental processes which in the cross-fertilized intact eggs is accomplished by the maternal nuclear material. Different results might be obtained if the sperm used were of a kind that reacted normally in the foreign egg cytoplasm, as would, for instance, be the case in crosses between Strongylocentrotus and Echinus (Baltzer, '10). It might be worth while to determine whether enucleated Strongylocentrotus eggs activated by Echinus sperm would produce plutei with recognizable parental characters. Other combinations furnishing the required conditions, viz., that the sperm chromosomes participate normally in mitosis in the egg cytoplasm, are possible.

## DISCUSSION

These observations offer, I believe, convincing proof of my earlier interpretation of the initial block to normal development in fish hybrids which, together with the increased number of crosses investigated cytologically, places on a surer footing our analysis of the factors involved in heterogeneric hybridization. The facts to be obtained from a study of such data may be most simply summarized by reference to tables 1 and 2.

TABLE 1

*Systematic position of the species crossed. Jordan and Evermann*(4)

| ORDER | SUBORDER | FAMILY, WITH NUMBERS OF FAMILIES INTERVENING | GENUS AND SPECIES |
|---|---|---|---|
| X. Haplomi | | 92. Poeciliidae | 300. Fundulus heteroclitus |
| | | 14 | |
| BB. Acanthopteri | 3. Percesoces | 106. Atherinidae | 356. Menidia menidia notata |
| | | 45 | |
| | 4. Rhegnopteri | 151. Sparidae | 551. Stenotomus chrysops |
| | | 9 | |
| | 7. Pharyngognathi | 160. Labridae | 627. Ctenolabrus adspersus |
| | | 24 | |
| CC. Plectognathi | 13. Craniomi | 184. Triglidae | 797. Prionotus carolinus |

Table 1 gives the systematic position of the species used in these crosses. Table 2 lists the crosses studied in the order of their width, that is, the crosses between the most closely related species are first and the most distantly related pair is last on the list. The width of cross is estimated by the number of families intervening between the two families to which the crossed pair belong and these numbers are placed in column 2 after the cross to which each applies. The families are numbered according to Jordan and Evermann(4). Column

3, table 2, gives the character of mitosis in each cross, and column 4, the grade of development that is reached according to the classified list appended to table 2. This list is based

TABLE 2

*Crosses in order of their width with mitotic and developmental results*

| 1. CROSS | 2. WIDTH OF CROSS. (SEE TEXT) | 3. CHARACTER OF MITOSIS | 4. GRADE OF DEVELOPMENT |
|---|---|---|---|
| 1. Stenotomus chrysops ♀ × Ctenolabrus adspersus ♂ | 9 | Abnormal | 2 |
| 2. Ctenolabrus adspersus ♀ × Stenotomus chrysops ♂ | 9 | Normal | 6 |
| 3. Fundulus heteroclitus ♀ × Menidia menidia notata ♂ | 14 | Normal | 5 |
| 4. Menidia menidia notata ♀ × Fundulus heteroclitus ♂ | 14 | Normal | 5 |
| 5. Ctenolabrus adspersus ♀ × Prionotus carolinus ♂ | 24 | Normal | |
| 6. Prionotus carolinus ♀ × Ctenolabrus adspersus ♂ | 24 | Normal | |
| 7. Menidia menidia notata ♀ × Ctenolabrus adspersus ♂ | 54 | Abnormal | 5 |
| 8. Ctenolabrus adspersus ♀ × Menidia menidia notata ♂ | 54 | Normal | 4 |
| 9. Fundulus heteroclitus ♀ × Stenotomus chrysops ♂ | 59 | Normal | 3 |
| 10. Fundulus heteroclitus ♀ × Ctenolabrus adspersus ♂ | 68 | Abnormal | 5 |
| 11. Ctenolabrus adspersus ♀ × Fundulus heteroclitus ♂ | 68 | Normal | 2 |
| 12. Menidia menidia notata ♀ × Prionotus carolinus ♂ | 78 | Abnormal | |
| 13. Fundulus heteroclitus ♀ × Prionotus carolinus ♂ | 92 | Abnormal | |

*Appendix to table 2*

Hybrid types graded according to the most advanced stage reached by any individual in the cross

1. Embryos succumb at the end of the cleavage period.
2. Embryos undergo a varying degree of notogenesis, but do not complete the closure of the germ ring.
3. Embryos in which concrescence is completed, but which fail to establish a circulation.
4. Embryos in which the circulation becomes established, but which fail to hatch.
5. Hatching embryos are very rare.
6. Hatching embryos are relatively frequent.

chiefly on the descriptions of the developmental results of many different teleost crosses given by Newman (8). From these tabulations as a starting-point it will be easier to define the variants which cytological studies have demonstrated in these heterogeneric crosses.

In the first place, such crosses fall into two large groups differing from each other in the type of their nuclear behavior during mitosis. So far, division into two categories has been marked; a study of many more crosses may show that there are border-line cases that are difficult to classify. These we would expect to be few in number compared to the total. The fact that we were able to predict the mitotic behavior in the Menidia and Prionotus cross on the basis of what was known of the results of other crosses with these species also lends support to this idea that the earliest known effect in hybrid eggs is a function of the cytoplasm. The terms fluidity, viscosity, surface tension, immediately suggest themselves, but a more specific definition of the cytoplasmic condition that interferes with the movements of the male chromosomes must await further analysis of the forces operating in nuclear and cell division.

Cytological studies on the elimination of chromatin in heterogeneric hybrids do not support the idea that the decrease in male chromatin in these early stages is due to its non-synthesis, as suggested by Guthrie(3). We have here a physical extrusion of paternal chromatin that is stainable and quite visible. Of this the figures of Balzer(1) and Matsui(5) as well as my own are undeniable evidence. Furthermore, the rate at which these bodies are synthesized is the rate at which the synthesis of the egg chromosomes occurs. The significance of this is still obscure, but if the synthesis is due to a specific enzyme carried in by the sperm, as Miss Guthrie assumes, some influence, undoubtedly originating in the egg, is affecting its activity.

These two fundamental classes of hybrids referred to above are further differentiated into several groups according to their fate after early cleavage, groups which correspond to the types included in the appendix to table 2. From that list it will be noted that some crosses cease development at the first of these stages; some crosses include the entire series in their progeny; others include only the first two, or three, or four of these embryonic types. There are, of course, intermediates in all of the crosses. The fate of any cross cannot be predicted from its earlier nuclear history. All of the types listed may result from either of the first two classes of hybrids. This seems to point to a second differential factor or set of factors quite independent of the first, and the question of the identity of these is apparently a two-fold one. There is no correlation between mitotic behavior and these developmental types. The stage is set differently in each of the two primary classes of eggs for subsequent events, and yet each class may give similar results.

Is there any differentiating factor which, when taken together with the mitotic behavior, will give a combination of factors that can be correlated with these several types of hybrid development? There seems to be. For instance, there are closely related pairs which when nuclear behavior is normal give embryos of advanced grade. Crosses 2, 3, and 4 are examples. On the other hand, distantly related pairs with the same normal division figures do not proceed very far in their development, as is the case in crosses 9 and 11. Clearly, the differential factor here is one of nuclear relationship, and a survey of table 2 emphasizes this interpretation.

There the crosses with normal mitosis very definitely show a decrease in the grade of their development from the top of the column toward the foot, while with the crosses in which abnormal mitosis occurs there is, on the contrary, an increase in developmental success, though not so marked, owing to the smaller number of cases, from above downward. The evidence from such a tabulation may claim, I believe, a considerable degree of validity, in spite of the fact that we have no assured criterion to go by in estimating quantitatively either the relationship between species or the steps in embryonic growth. The fact that a single cross shows a variable progeny complicates the task of determining the degree of its developmental success. Nevertheless, I regard the stages as listed in the appendix to table 2 as fair approximations and their use in this connection permissible.

My data have been taken entirely from heterogeneric crosses, but the homogeneric crosses between two species of Fundulus which Miss Guthrie(3) advances as an objection to the idea outlined here presents no facts at variance with this explanation. The reciprocal crosses between F. heteroclitus and F. majalis are both relatively successful, one hatches and the other develops up to the time of hatching, exactly as we would expect in such closely related species *if early division processes were normal.* Theoretically, there is no reason to expect that the mitotic divisions in the homogeneric Fundulus hybrids would be in any way abnormal merely because the behavior of their cytoplasmic inclusions is similar to that in another cross in which nuclear behavior is known to be abnormal. The successful homogeneric cross, F. heteroclitus ♀ × F. majalis ♂ does not belong in the same category with the heterogeneric hybrid between Fundulus and Ctenolabrus. In the first place, the number of hatching embryos in the interspecies cross is large compared to the very few hatching individuals reported from the other cross, and, in the second place, the hatching embryos of the former cross show to an appreciable degree a blend of parental characters in contrast to the purely maternal characters of the wider cross(2, 7). Miss Guthrie seems not to have drawn these necessary distinctions and, furthermore, she makes a mistake in attempting to correlate abnormal mitosis with the upset in cytoplasmic equilibrium that she finds in some of the crosses she studied. The former is an expression of a general cytoplasmic property, essentially the same in all eggs, but differing in degree, which affects the movements of the male chromosomes, while the latter condition, an abnormal accumulation of cytoplasmic inclusions, is due, according to her interpretation, to foreign enzymes which alter the rate of the transformation of materials stored in the cytoplasm. The former is a physical effect which is not highly specific, since it exhibits only two phases; the latter is a chemical reaction which is highly specific and which varies in every cross studied. Undoubtedly, both of these events, the character of the early mitosis and the manner in which metabolic processes are altered in the egg, are factors in determining the development of hybrid eggs. Both must be included in any complete analysis, but this cannot be forthcoming as long as there is any doubt about the essential details of either process.

I have already pointed out that if the paternal chromatin is diminished in the crosses in which abnormal division prevails, it is not due to a failure in the earlier stages of the egg to synthesize chromatin, but is due rather to a physical elimination of the paternal chromatin entities. Synthetic processes may play a later rôle. I have discussed elsewhere(10) the probable failure of the Ctenolabrus egg to synthesize the necessary chromatin, basing my remarks not only on a result of the outcome of that cross, but on a study of the nuclei of late blastoderms of normal and cross-fertilized eggs. The pure-bred blastoderms showed binucleolate nuclei. The hybrid blastoderms, on the other hand, showed nuclei with only one nucleolus. There was a slight difference in the ages of these eggs which may nullify their bearing on this point, but we are warned by the suggestiveness of such observations of the complexity of the problem. The analysis of the cytoplasmic inclusions in the egg of Ctenolabrus, either self- or cross-activated, is too inadequate to warrant any conclusion as to their significance in the Ctenolabrus crosses.

The extended list of crosses presented in table 2 justifies a reemphasis of the statement that nuclear relationships play a rôle in heterogeneric hybrids and must be reckoned with. A more definite expression of the nature of this interaction is not yet in order, but these observations permit us to point out here a possible connection between the early mitotic disturbance and the later interaction of the germ plasms. Newman's cross between Fundulus and Scomber(9) led him to the following statement (p. 416):

> These experiments may be taken to indicate that in the heterogeneric crosses no harmonious structural differentiation can result without the neutralization or elimination of the disharmonious paternal materials.

My observations suggest that the mechanism of neutralization of the foreign sperm is accomplished by the elimination of the paternal chromatin, although they cannot be regarded as establishing the fact. More detailed study of later stages than are dealt with here are necessary before an ultimate proof can be realized.

The cross described in this paper adds evidence to my former observations as to the variation in amount of the chromatin eliminated during early cleavage, and this seems to me to bear on the question as to what it is that causes such a diversity in the products of a single cross. It is a matter of common observation that when one cross is made not every egg begins development, and among those that do many die during cleavage. If the cross is one that proceeds beyond gastrulation, there is a succession of mortalities in the survivors. In certain crosses the series obtained runs the gamut from those eggs that cease development in the early cleavage stages to those that hatch normally, and all of these come from a combination of similar eggs and sperm. These facts taken together add force to the following suggestion(10, p. 257):

> Assuming that the entrance of the spermatozoon into the egg and the movements of the chromosomes during cell division are comparable processes in that they have their cause in changes in surface tension, though varying in complexity and perhaps in degree of specificity, it is conceivable that slight variations in the conditions of eggs and sperm which were no hindrance to fertilization might cause a disturbance in the more complex or more specific mechanism of mitosis. This would account for both individual variations *within one cross* as well as for the different results obtained at different times *in the same* cross. (The italics were not used in the original paper.)

This is what was meant by my earlier phrase "the variable specificity of the effect of the cytoplasm toward the foreign spermatozoon"(10, p. 261). Its relation to the two more fundamental factors discussed above has been misunderstood by Miss Guthrie(3). All that is claimed for it is that it has a modifying influence on the initial cytoplasmic effect.

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# PLATES

All of the figures, with the exception of figure 8, were made with the aid of a camera lucida at a magnification of 2600 diameters. The drawings were finished with the objects under close observation at a lower magnification and reproduced as drawn.

## PLATE 1

### EXPLANATION OF DRAWINGS

1, a and b Two consecutive sections of an early anaphase spindle. First cleavage. The chromosomes in 1a were too crowded to include them all in the drawing. Pairs of daughter chromosomes were easily identified and contrasted markedly with the retarded elements lying at the equator and to the left of the spindle. The section of the spindle shown in figure 1b contained only four definite pairs of chromosomes.

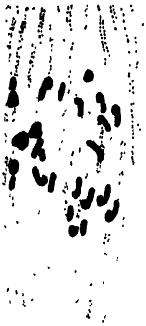
2 Midanaphase of second cleavage. Extensive lagging. Only one section drawn.

3 Late anaphase spindle of the third cleavage. Of the affected chromatin bodies, four lie at the equator and will probably be eliminated. One undivided chromosome is mounting to the upper pole of the figure.

4 Midanaphase of the first cleavage. One-sided lagging, but no elimination.

5 A later anaphase of the second cleavage. Definite lagging, but not extensive. No undivided chromosomes on this spindle.

6 One section of a late anaphase, showing lagging, non-disjunction, and elimination.



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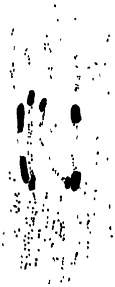

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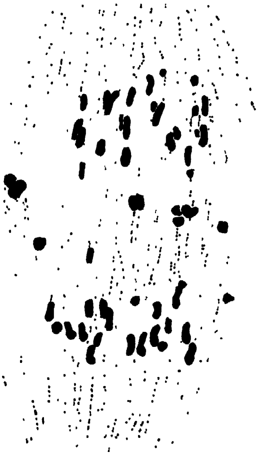

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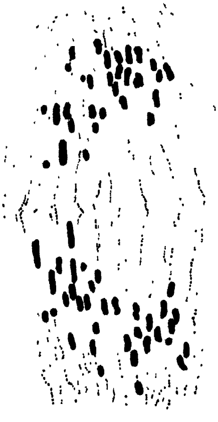

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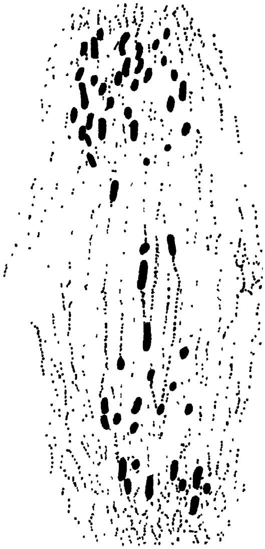

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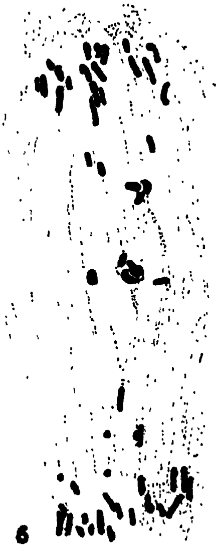

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## PLATE 2

### EXPLANATION OF DRAWINGS

7, a and b Two sections of a midanaphase of the second cleavage. In the other cell of this egg abnormal features were present, but less marked.

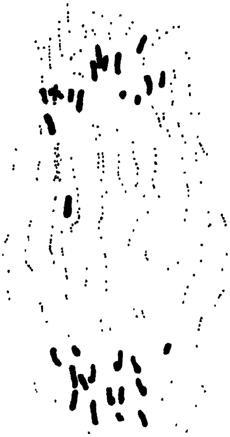
8 Late telophase of the third cleavage. Chromosome vesicles indistinct. Eliminated chromosomes lying in the cleavage plane. Similar remains were observed in other cells of this egg.

9 Part of a spindle in the late telophase. The chromosomes at the poles are becoming vesicular and are barely discernible. The eliminated chromosomes at the equator are very clear. One other such body was present, but has been omitted from the drawing.

10 A very early anaphase of the second cleavage. This is the first stage in which the mitotic abnormality shown in these figures could be recognized.

11 One section of a second-cleavage spindle in which all but one of the chromosomes were drawn into the polar groups.

12 A very late anaphase in which the amount of affected chromatin is small. None was seen in the other two sections of this spindle.



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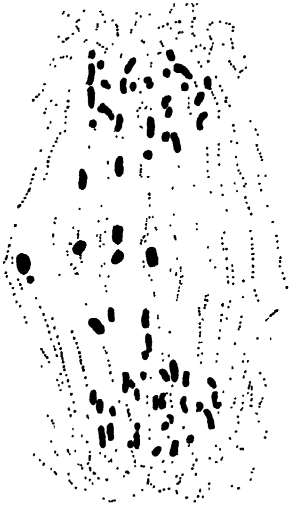

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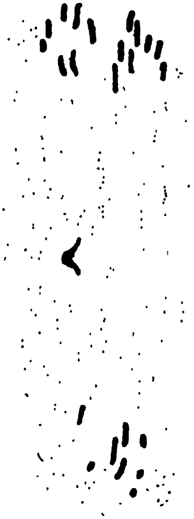

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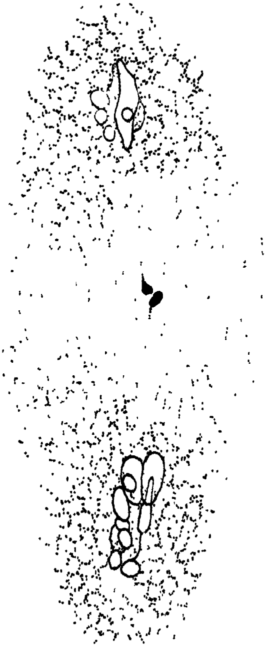

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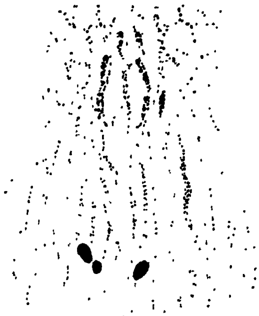

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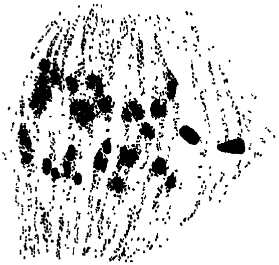

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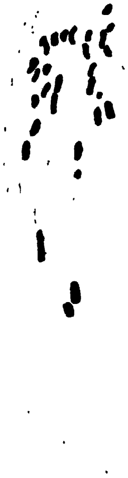

12

# VARIATIONS IN CHROMOSOME NUMBER IN THE SPERMATOGENESIS OF PHILOSAMIA CYNTHIA

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TWO PLATES (TWENTY-SEVEN FIGURES)

AUTHOR'S ABSTRACT

A study of spermatogenesis was made on four groups of a pedigreed strain of the moth Philosamia cynthia, and the results were compared with spermatogenesis in the wild material.

Deviations from the normal number of chromosomes were observed in two groups. In some individuals the haploid number was 12 instead of 13. Giant spermatocytes were also observed with twenty-four chromosomes In other individuals, two haploid numbers, 13 and 14, occurred in the same testis.

The twelve-chromosome condition is due to linkage of two chromosomes during the late prophase of the primary spermatocyte. The double chromosomes thus formed appeared to divide equally in both divisions. The origin of the fourteenth chromosome was not determined.

Correlation of the genetic and cytological data indicates the restriction of aberrant chromosome complexes to two of the four groups and the regularity of the twelve-chromosome complex in certain families, suggesting the conclusion that a new strain arose in the pedigreed material with regard to chromosome variability and that the twelve-chromosome condition is a well-defined characteristic, partly established in some families and probably fully established in others.

## CONTENTS

Introduction .......... 600
Scope of the paper .......... 600
Cytological observations .......... 600
  A. Normal spermatogenesis .......... 600
  B. Spermatogenesis in individuals with 12 as the haploid number .......... 601
  C. Giant cells .......... 602
  D. Spermatogenesis in individuals with 13 and 14 as the haploid number. 604
Pedigree of groups I to IV and the relation to them of chromosome deviations .......... 604
  A. Pedigree of groups I to IV .......... 604
  B. Distribution in groups and families of normal and abnormal chromosome numbers .......... 605
  C. Correlation between the genetic record and the cytological results.. 606
Discussion of the relation of chromosome number to species. Conclusion ... 607
Summary .......... 609
Bibliography .......... 609

## INTRODUCTION

The following observations were made upon pupae of the moth Philosamia cynthia, given to the writer some years ago by Prof. Henry E. Crampton, from some material he was rearing in a study of pupal characters as a basis of selection. The possibility was suggested that there might be some chromosomal differences observable during spermatogenesis between the pedigreed strain and the wild material.

The pupae used in this study were from thirty-four different families, comprising part of four groups, whose history will be given later. The testes were dissected out, sectioned, and stained according to methods described by the writer in an earlier paper ('07).

## SCOPE OF THE PAPER

An examination of metaphase groups of chromosomes in the first and second spermatocyte divisions revealed the following facts:

1. The normal haploid number of chromosomes (13) was present in both first and second spermatocyte metaphases in all families studied in groups II and IV.

2. In groups I and III deviations from the normal number were observed in both spermatocyte divisions. Some individuals in one family, and all the material available from three families, were characterized by 12 chromosomes instead of 13. In another family certain individuals showed 12 chromosomes, others both 13 and 14 in the same testis.

The scope of this paper comprises two problems: first, the cytological history of the chromosomes; second, the correlation of the chromosomal deviations with the known history of the material.

## CYTOLOGICAL OBSERVATIONS

### *A. Normal spermatogenesis*

A review of the normal features of spermatogenesis will facilitate an inquiry into the origin of the aberrant conditions noted above:

1. The spermatogonia contain twenty-six chromosomes of approximately the same size and shape (fig. 1).
2. There is a definite spireme with a simple plasmosome. The spireme segments into thirteen parts; twelve form rings, the thirteenth becomes attached to the plasmosome as a chromatin mass (fig. 2).
3. The chromatin mass represents a sex chromosome which is bivalent like the rings. (This sex chromosome was originally interpreted by the writer as an equal X-Y pair, as in Nezara (Wilson, '05), but researches on oogenesis in lepidoptera by Doncaster ('14) and by Seiler ('14) show that the female is the digametic sex. The equal sex chromosomes in the male would therefore be homologous to an XX pair or, to use a more recent terminology, a ZZ pair.)
4. During the first spermatocyte prophase, the rings condense to form twelve rounded chromosomes, and the sex chromosome also forms a mass of similar shape. Thus, thirteen chromosomes are present in the prophase, the sex chromosome being at this time indistinguishable from the others (fig. 3).
5. First and second metaphases show thirteen chromosomes. Divisions are equal so that spermatids receive similar chromosome groups.

### *B. Spermatogenesis in individuals with 12 as the haploid number*

No differences from the normal sequence of chromatin changes were observable until the late prophase of the primary spermatocytes. The thirteen chromosomes, derived from the twelve rings and the sex chromosome, are visible in the early prophase as separate masses (fig. 3), but in the later prophase only twelve chromosomes are observable (fig. 4), one of them appearing bipartite, and obviously representing two chromosomes adhering together. In the following figures the double chromosome is indicated by *D*. Complete counts of first metaphase show invariably twelve chromosomes, and there is abundant evidence of this double body in both polar and side views (figs. 5 to 9). Its position was always at the periphery of a chromosome group (figs. 5 to 13).

Whether this linkage involves the sex chromosomes or two automosomes is a matter of conjecture, for the sex chromosome cannot be identified except during the growth period. In many cases the components appeared to be slightly different in size. Figure 7, a side view of part of a first metaphase, indicates the typical position of the double chromosome, its long axis being at first placed transversely to the spindle.

The sequence of events from this point on is interpreted to be as follows: One component is pulled out toward the poles of the spindle, the other appearing as a knob or enlargement in the middle (figs. 8, 9). Eventually this, too, becomes divided, apparently equally, and the products of the division may be identified as elongated bodies at each end of the spindle (figs. 10 to 13). A lagging in division was frequently noted.

Second metaphase groups were in all respects similar to the first, except in size (figs. 15, 16). Figures 17 to 21 show side views of the double chromosome during the second division. Its general similarity in form to that of the early first metaphase may be noted in figures 17 to 20. The two ends of the chromosome are interpreted, as before, to be one component divided, the middle region the other component, undivided. Figure 21 shows this one also separated into two parts.

All the evidence points in favor of an equal division of the double chromosome. Telophase counts were difficult to make, owing to the small size of the chromosomes, but a few oblique polar views showed clearly twelve at each pole (figs. 22, 23). The double chromosome could not be identified at this stage.

### C. Giant cells

The occurrence of giant spermatogonia in testes fixed in the late spring, differing from normal ones only in size, was reported by the writer in an earlier paper ('07). They were also observed in the pedigreed material. In testes of the twelve chromosome groups there were noted in addition giant spermatocytes in the metaphase stage of both divisions. Unlike the large spermatogonia which contained twenty-six chromosomes, these giant cells showed equatorial plates with twenty-four chromosomes, including two which appeared double (fig. 14). That these cells are really spermatocytes is evidenced by the fact that they were present in the same cysts with cells containing twelve chromosomes.

The growth period was studied in an endeavor to discover the origin of these large cells, but no stages were found with more than the normal number of chromatin rings. It seems probable that these cells arose by the fusion of cells in pairs during the late first prophase, at the time of the rupture of the nuclear wall and after the double chromosome had formed, thus freeing twenty-four chromosomes in the cytoplasm. Figure 14, a polar view of a giant metaphase, may be interpreted on this basis, the group being readily separable horizontally into two, each with twelve chromosomes, including a double one. If such a union occurred, it must have been before the time of spindle formation, for single normal spindles were present in the giant cells with chromosomes arranged regularly upon them. By whatever means the duplicated chromosome group was established in the primary spermatocytes, the cells apparently divided regularly, for giant secondary spermatocytes were present, likewise with twenty-four chromosomes.

Since later stages of the giant cells were lacking, no conclusion may be drawn as to whether these cells were potential giant sperms such as are known to occur in many groups of insects. Dimegalous sperms have been described as originating through the suppression of spermatocyte divisions, or the reuniting of both nucleus and cytosome after division (Wilson, '25). The dimegaly described above is due to neither of these causes, since, as we have seen, it is already apparent in the first metaphase stage. Its origin may be, as suggested, through the fusion of primary spermatocytes in the prophase stage.

### *D. Spermatogenesis in individuals with 13 and 14 as the haploid number*

In the case now to be described, a discrepancy in chromosome number was observed within the same testis. This condition was found in three testes, in one family only. In certain cysts of primary spermatocytes in prophase, the cells contained fourteen chromosomes (figs. 24, 25); in other cysts the normal number appeared. First metaphase plates with fourteen chromosomes (fig. 26) ranged in each slide from about one-fourth to three-fourths of the total number counted. Second metaphase plates with fourteen (fig. 27) were about one-fourth of the total counted. The origin of the fourteenth chromosome could not be ascertained, owing to the lack of sufficient stages. It may be that the increase in number is due to fragmentation, arising probably not before the early prophase, but it is impossible to detect which chromosome is the fragment.

## PEDIGREE OF GROUPS I TO IV AND THE RELATION TO THEM OF CHROMOSOME DEVIATIONS

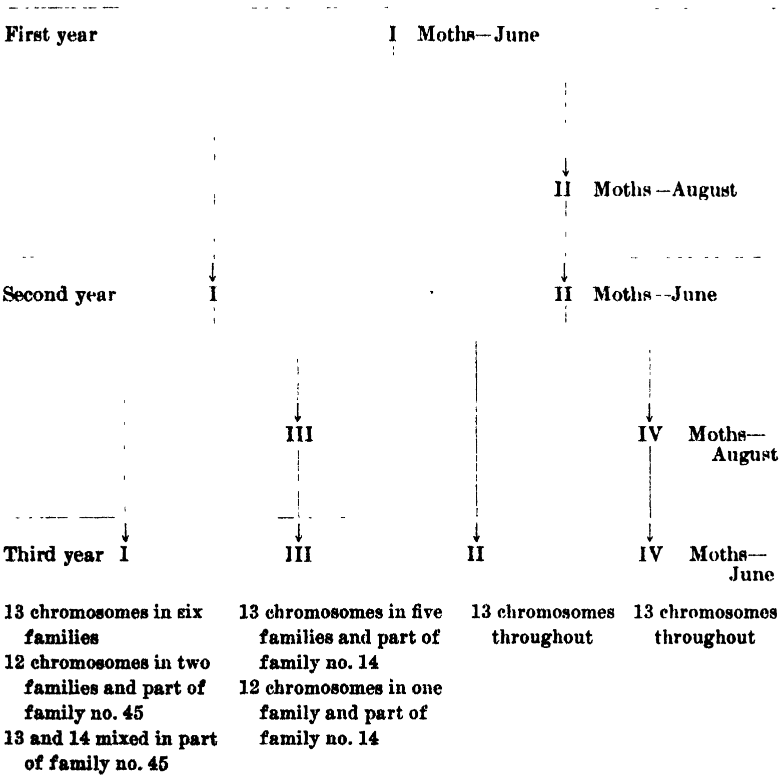
### *A. Pedigree of groups I to IV*

I am indebted to Doctor Crampton for his generosity in placing at my disposal the following diagram and genetic data, from unpublished records covering a period of three years. To these I have added data regarding chromosomes, which will be referred to later.

A subdivision of the pupae produced from eggs of the original wild material was made during the first year of the experiment, when it was discovered that some pupae were transformed into moths during August instead of the following June. These moths were numbered group II. Their descendants metamorphosed the next June, at the same time as the single brooded lot, group I. During the second year, group II was subdivided, also on the basis of single- and double-brooded conditions, both of which appeared. The double-brooded lot was designated as group IV; the single-brooded, group II of the third year.

Group I of the second year, like group II of that year, also split into single- and double-brooded lots. The single-brooded ones were numbered group I of the third year, and the double-brooded, group III. Pupae from these four groups formed the material used in this study.

*Diagram showing the pedigree of groups I to IV and the relation to them of chromosomal variations*


First year
I Moths—June
II Moths—August
Second year
I
II Moths—June
III
IV Moths—August
Third year I
III
II
IV Moths—June
13 chromosomes in six families
12 chromosomes in two families and part of family no. 45
13 and 14 mixed in part of family no. 45
13 chromosomes in five families and part of family no. 14
12 chromosomes in one family and part of family no. 14
13 chromosomes throughout
13 chromosomes throughout

### *B. Distribution in groups and families of normal and abnormal chromosome numbers*

A detailed survey of material from ten families in group II and eight families in group IV revealed that chromosome numbers here were entirely normal, no aberrant conditions being observed in hundreds of metaphases.

An analysis of chromosomal conditions in groups I and III is presented in the accompanying table, which includes also the amount of material used and the approximate number of chromosome counts made. The salient features may be indicated as follows:

1. The normal haploid number of thirteen chromosomes is present in six families in I and in five families plus part of family 14 in III.

*Table showing the distribution of chromosome numbers in spermatocyte metaphases in groups I and III*

| GROUP | NUMBER OF CHROMOSOMES PRESENT | NUMBER OF FAMILIES | NUMBER OF TESTES EXAMINED | NUMBER OF METAPHASES COUNTED | |
|---|---|---|---|---|---|
| I | 13 | 6 | 10 | 128 | |
| III | 13 | 5 and part of family 14 | 13 | 127 | |
| I | 12 | 2 and part of family 45 | 10 | 269 | |
| III | 12 | 1 and part of family 14 | 4 | 186 | |
| I | 13 and 14 in the same testis | Part of family 45 | 3 | 13 chromosomes | 14 chromosomes |
| | | | | 139 | 56 |

2. The twelve-chromosome condition is present in two families plus part of family 45 in I, and in one family plus part of family 14 in III.

3. The presence of thirteen and fourteen chromosomes within the same testis is characteristic of part of family 45 in I. This is the only case where deviation was observed within the individual.

### C. Correlation between the genetic record and cytological results

Tracing, now, on the diagram the connection between chromosome numbers and the genetic record, it will be seen that all individuals descended from the original group II are entirely normal as regards chromosome numbers. In groups I and III lie all the divergences described. It will be noted that both the normal and the twelve-chromosome condition is characteristic of both these groups, with a preponderance of the normal condition in each group. The greatest observed variability is in group I, due to the presence in family 45 of twelve chromosomes in some individuals and both thirteen and fourteen in others. The even-numbered groups thus show a stable chromosome complex; the odd-numbered groups are characterized by a certain amount of chromosome variability.

## DISCUSSION OF THE RELATION OF CHROMOSOME NUMBER TO SPECIES. CONCLUSION

The processes by which chromosome numbers have been changed in different species is believed to be in some cases by linkage of chromosomes, and by the appearance of supernumeraries, including among the latter chromosomes derived by fragmentation (Wilson, '25). Linkage between autosomes and between sex chromosomes and autosomes has been described in a number of species, notably by McClung ('17) on Orthoptera and Seiler and Haniel ('21) on Lepidoptera. In Philosamia cynthia the similarity of the X chromosome to the autosomes renders it impossible to determine the type of linkage here.

It is known that linkage may be permanent as in the multiple chromosomes described by McClung, or temporary as described by Seiler and Haniel in Lymantria. In this form linkage occurred in the male between the spermatogonia and the first spermatocyte division, and later in the female; but the linkage was resolved after fertilization, since the blastoderm had the diploid number of chromosomes. The origin of linkage in Philosamia, as already indicated, was found to be in the late first spermatocyte prophase, but the question of its permanence must remain an open one, since, unfortunately, the rearing of the moths was terminated before the cytological study was made. The same situation occurs in regard to the question of permanence of the fourteen chromosome condition.

Permanent linkage leads presumably to differences in chromosome number in different species. Harvey ('20) states that there is considerable evidence from certain groups of insects that numbers change by fusion and by splitting of chromosomes. She has described chromosome complexes in three species of Notonecta ('10), suggesting the interpretation that two of them were derived from a third by a process of progressive fusion. In these cases there was, naturally, a correlation between the species characteristics and the chromosomal structure, but the point is made that the species differences might have been due to changes in other chromosomes than these showing splitting or fusion. Thus, the occurrence of chromosomal differences does not necessarily have a causal relation to species differences, and, conversely, species changes may arise without any observable changes in the chromosomes. A good example of this is to be seen in Yatsu's work ('13) on the spermatogenesis of the silkworm. He found no differences in chromosome numbers or characters in seventeen varieties of Bombyx mori, and he concluded that there was in this form no morphological correlation between external features and chromosomes.

It may be questioned, then, whether further breeding of aberrant families of Philosamia cynthia, also a lepidopteran and generally similar to Bombyx in chromosome form, would have disclosed any correlation between the chromosomal changes and somatic differences. Two points, however, stand out clearly: first, the restriction of the aberrant condition to groups I and III; secondly, the regularity of the twelve-chromosome condition within certain families in these groups. Two conclusions are drawn from this, valid at least for the period of the experiment: first, that there has arisen a new strain with regard to chromosome variability; secondly, that the twelve-chromosome condition derived by linkage is not fortuitous, but is a well-defined characteristic, partly established in some families and probably fully established in others.

## SUMMARY

1. A study of spermatogenesis was made on four groups of a pedigreed strain of Philosamia cynthia, and the results were compared with spermatogenesis in the wild material.

2. Deviations from the normal number of chromosomes were observed in two groups. In some individuals the haploid number was 12, instead of 13. Giant spermatocytes were also observed with twenty-four chromosomes, including two double ones. In other individuals two haploid numbers, 13 and 14, occurred in the same testis.

3. The twelve-chromosome condition is due to linkage of two chromosomes during the late prophase of the primary spermatocyte. The double chromosome thus formed appeared to divide equally in both divisions. The origin of the fourteenth chromosome was not determined.

4. Correlation of the genetic and cytological data indicates the restriction of aberrant chromosome complexes to two of the four groups, and the regularity of the twelve-chromosome complex in certain families. These facts suggest two conclusions: first, that a new strain arose in the pedigreed material with regard to chromosome variability; secondly, that the twelve-chromosome condition is a well-defined characteristic, partly established in some families and probably fully established in others.

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## DESCRIPTION OF PLATES

All the figures are from camera drawings made with compensating ocular no. 8 with a tube length of 160 mm., and $\frac{1}{12}$ oil-immersion lens. They were enlarged 2½ diameters with a drawing camera, corrected from the original, and then reduced to ⅔ in the final plates.

### PLATE 1

#### EXPLANATION OF FIGURES

Spermatogenesis in individuals with 12 as the haploid number of chromosomes.

1 Metaphase of late spermatogonial division, polar view, showing the normal number of chromosomes.

2 Growth period of primary spermatocyte, showing twelve chromatin rings and the sex chromosome (*X*) attached to the plasmosome (*P*), as in normal spermatogenesis.

3 Early prophase of primary spermatocyte. Thirteen separate chromosomes present, as in the normal.

4 Late prophase of primary spermatocyte, with twelve chromosomes. Double chromosome shown at *D*.

5 to 14 First spermatocyte division. Double chromosome at *D*.

5 and 6 Polar views of metaphase. Twelve chromosomes present.

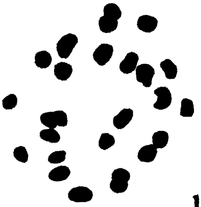
7 to 9 Parts of side views of metaphase, showing position of double chromosome on the spindle and beginning of division.

10 and 11 Anaphases in side view, showing characteristic form of double chromosome during division. The upper group in each figure is complete.

12 Double chromosome in telophase, side view.

13 Polar view of telophase, with twelve chromosomes.

14 Giant primary spermatocyte. Polar view of metaphase. Twenty-four chromosomes present, including two double ones.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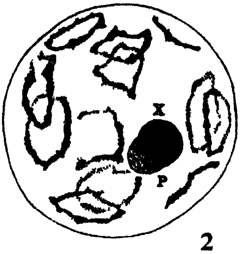

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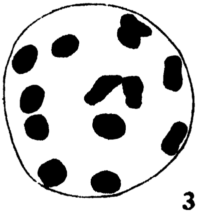
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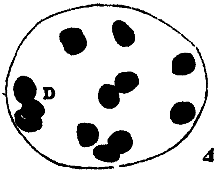

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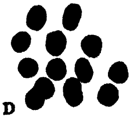

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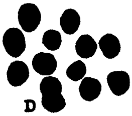

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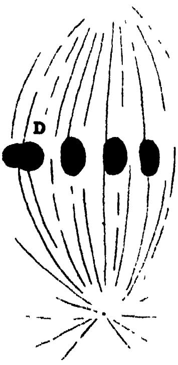

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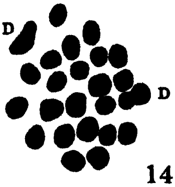

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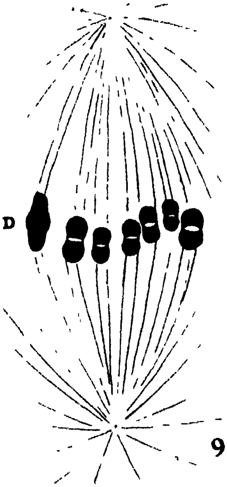

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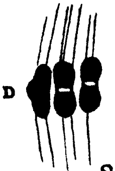

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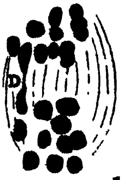

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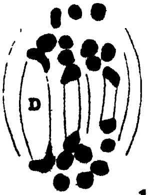

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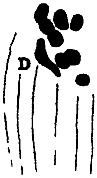

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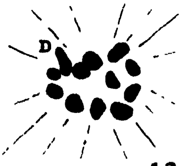


13

## PLATE 2

### EXPLANATION OF FIGURES

15 to 23 Second spermatocyte divisions in individuals with 12 as the haploid number of chromosomes.

15 and 16 Polar views of metaphase with twelve chromosomes.

17 Three side views of double chromosomes on spindle in metaphase. Tripartite form characteristic of this stage.

18 Section of a metaphase, side view, showing double chromosome and three others.

19 Side view of anaphase. Double chromosome lagging in division.

20 Double chromosome, tripartite, further drawn out on spindle, but middle region undivided.

21 Both parts of double chromosome divided equally, to be distributed to daughter cells.

22 and 23 Polar views of telophase, twelve chromosomes present. Double chromosome not identifiable in this stage.

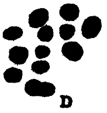
24 to 27 Spermatocytes with fourteen chromosomes, from individuals with 13 and 14 as the haploid number.

24 Prophase of primary spermatocyte. Plasmosome at *P*.

25 Later prophase of primary spermatocyte.

26 Polar view of metaphase, first spermatocyte division.

27 Polar view of metaphase, second spermatocyte division.



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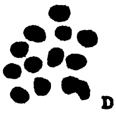

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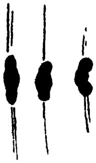

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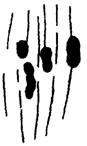

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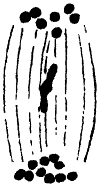

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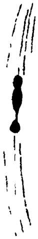

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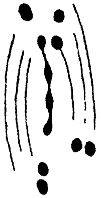

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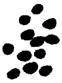

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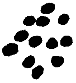

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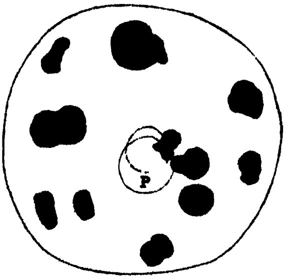

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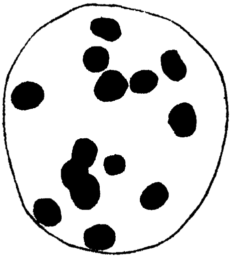

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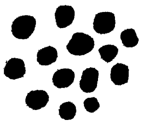

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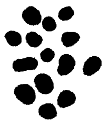

27

# INDEX

AMINO-ACIDS by Paramecium caudatum. The metabolism of . . . 555
Amoeba proteus. The effect of certain physical and chemical factors on locomotion and other life-processes in . . . 97
Amphibia urodela. The septomaxillary. I. In the . . . 441
Anatomy and life-history of a fresh-water mollusk of the genus Sphaerium. The . . . 473
Anurans. A study of the tonsillar developments in the lingual region of . . . 399

CAMBARUS virilis. The morphology and physiology of the hepatopancreas of . . . 505
Chromosome number in the spermatogenesis of Philosamia cynthia. Variations in . . . 599
Chromosomes in meiosis, fertilization, and cleavage in the grasshopper egg (Orthoptera) A study of the 47
Cleavage in the grasshopper egg (Orthoptera). A study of the chromosomes in meiosis, fertilization and . . . 47
Cranial musculature of the toadfish (Opsanus tau). The . . . 209

DEDERER, PAULINE H. Variations in chromosome number in the spermatogenesis of Philosamia cynthia . . . 599
Digital pads of the tree frogs A study of the phylogenesis of an adaptive structure The . 259
Discoglossus pictus Otth. Cytoplasmic constituents in the developing egg of . . . 233
DORMAN, HENRY P. The morphology and physiology of the hepatopancreas of Cambarus virilis . . 505
DORMAN, HENRY P. The morphology and physiology of the invertebrate liver and hepatopancreas . . . . 537

EGG of Discoglossus pictus Otth. Cytoplasmic constituents in the developing . . . 233
Egg (Orthoptera). A study of the chromosomes in meiosis, fertilization, and cleavage in the grasshopper . . . 47
EMERY, FREDERICK E. The metabolism of amino-acids by Paramecium caudatum . . . 555
Evolution: A discussion of data bearing upon 'orthogenesis.' Trends in . . . 1

FAT-BODY in Hymenoptera. Observations on the . . . 121
Fertilization, and cleavage in the grasshopper egg (Orthoptera). A study of the chromosomes in meiosis . . . 47
Frogs. A study of the phylogenesis of an adaptive structure. The digital pads of the tree . . . 259

GRASSHOPPER egg (Orthoptera). A study of the chromosomes in meiosis, fertilization, and cleavage in the . . . 47

HEATH, HAROLD. A sexually mature turbellarian resembling Müller's larva . . 187
Hepatopancreas of Cambarus virilis. The morphology and physiology of the . . . 505
HIBBARD, HOPE. Cytoplasmic constituents in the developing egg of Discoglossus pictus Otth . . . 233
HOPKINS, D. L. The effect of certain physical and chemical factors on locomotion and other life-processes in Amoeba proteus . 97
Hybrids. I. The early mitotic behavior in the cross between Menidia menidia notata ♀ and Prionotus carolinus ♂. Developmental factors in teleost . 579
Hymenoptera Observations on the fat-body in . . . 121

JAECKLE, M. E. G. K NOBLE AND. The digital pads of the tree frogs A study of the phylogenesis of an adaptive structure . 259

LAPAGE, ENID OLDHAM. The septomaxillary. I. In the Amphibia urodela . . . 441
Larva. A sexually mature turbellarian resembling Müller's . . . 187
Life-history of a fresh-water mollusk of the genus Sphaerium. The anatomy and . . . 473
Lingual region of anurans. A study of the tonsillar developments in the . . . 399
Liver and hepatopancreas. The morphology and physiology of the invertebrate . . . 537
Locomotion and other life-processes in Amoeba proteus. The effect of certain physical and chemical factors on . . . 97

McNABB, JOSEPHINE WILLIS. A study of the chromosomes in meiosis, fertilization, and cleavage in the grasshopper egg (Orthoptera) . . . 47
Man. Spiral movement in . . . 293
Meiosis, fertilization, and cleavage in the grasshopper egg (Orthoptera). A study of the chromosomes in . 47
Menidia menidia notata ♀ and Prionotus carolinus ♂. Developmental factors in teleost hybrids. I. The early mitotic behavior in the cross between . . . 579
Metabolism of amino-acids by Paramecium caudatum. The . . . 555
METCALF, MAYNARD M. Trends in evolution: A discussion of data bearing upon 'orthogenesis' . . 1

Mitotic behavior in the cross between Menidia menidia notata ♀ and Prionotus carolinus ♂. Developmental factors in teleost hybrids. I The early ................ 579
Mollusk of the genus Sphaerium. The anatomy and life-history of a fresh-water .................. 473
MONK, CECIL R. The anatomy and life-history of a fresh-water mollusk of the genus Sphaerium ... 473
Morphology and physiology of the hepatopancreas of Cambarus virilis. The ................ 505
Movement in man. Spiral ........ 293
MYERS, MABEL A. A study of the tonsillar developments in the lingual region of anurans ........ 399

NOBLE, G. K., AND M. E. JAECKLE. The digital pads of the tree frogs. A study of the phylogenesis of an adaptive structure ............ 259

(OPSANUS tau). The cranial musculature of the toadfish . . 209
'Orthogenesis.' Trends in evolution: A discussion of data bearing upon .................. 1
(Orthoptera). A study of the chromosomes in meiosis, fertilization, and cleavage in the grasshopper egg .................. 47

PANKRATZ, DAVID S. The cranial musculature of the toadfish (Opsanus tau) .......... 209
Paramecium caudatum. The metabolism of amino-acids by . . . . 555

Philosamia cynthia. Variations in chromosome number in the spermatogenesis of .............. 599
Phylogenesis of an adaptive structure. The digital pads of the tree frogs. A study of the .............. 259
Physiology of the hepatopancreas of Cambarus virilis. The morphology and .................... 505
PINNEY, EDITH. Developmental factors in teleost hybrids. I. The early mitotic behavior in the cross between Menidia menidia notata ♀ and Prionotus carolinus ♂ ... 579
Prionotus carolinus ♂. Developmental factors in teleost hybrids. I. The early mitotic behavior in the cross between Menidia menidia notata ♀ and .................... 579

SCHAEFFER, A. A. Spiral movement in man ............... 293
SCHMIEDER, RUDOLF G. Observations on the fat-body in Hymenoptera. 121
Septomaxillary. I. In the Amphibia urodela. The .......... .... 441
Spermatogenesis of Philosamia cynthia. Variations in chromosome number in the .............. 599
Sphaerium. The anatomy and life-history of a fresh-water mollusk of the genus ... . .......... 473

TOADFISH (Opsanus tau). The cranial musculature of the . ... 209
Tonsillar developments in the lingual region of anurans. A study of the .......... ... ...... 399
Turbellarian resembling Müller's larva. A sexually mature .......... 187

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